Novel synthetic approaches to phosphonate analogues of fructose phosphates

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Award date:
1991

Awarding institution:
University of Bath

NOVEL SYNTHETIC APPROACHES TO PHOSPHONATE ANALOGUES OF FRUCTOSE PHOSPHATES

Submitted by Gavin David Heffernan

for the degree of Ph.D.

of the University of Bath

1991

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ACKNOWLEDGEMENTS

I would like to thank my supervisor, Prof. Malcolm Campbell for his friendship, advice and constant encouragement throughout this project. I am indebted to my industrial supervisor, Dr. Terry Lewis of I.C.I. Agrochemicals, for his helpful discussions and support.

My appreciation goes to the technical staff at the University of Bath for their invaluable services. Special thanks are due to Mr. Dave Wood and Mr. Harry Hartell (^{1}H and ^{13}C nmr), Mr. Alan Carver (elemental microanalysis), Mr. Chris Cryer (mass spectroscopy), Mrs Sue Boucher, Mr. John Bradley and Mr. Russel Barlow (organic stores), and, of course, June and Freda for all the washing up.

Thanks are also due to Dr. Mary Mahon and Dr. Kieran Molloy for the X-ray crystal structure determination.

I would also like to thank all my colleagues in the organic department for their good humour throughout my time in Bath.

For the skilful typing of the experimental I am indebted to Mrs Jo Curtis.

Finally, I am grateful to the SERC and to I.C.I. Agrochemicals for providing a CASE studentship.

To my parents

Summary

The work described in this thesis is introduced by an account of the methods currently available for synthesising carbohydrate phosphonates. This is followed by a review of previous synthetic efforts towards phosphonate analogues of D-fructose phosphates.

An alternative route to carbohydrate phosphonates was envisaged *via* phosphorus ring-opening of epoxides. Methodology was developed for the synthesis of novel spiro-anomeric epoxides. The acid and base catalysed epoxide ring-opening reactions were investigated with a variety of heteroatom nucleophiles. The base catalysed reaction was found to occur exclusively at the least hindered carbon atom, yielding diastereoselectively the β-anomer. The β-siloxy diethyl phosphonate produced on epoxide ring opening with diethyl trimethylsilyl phosphite was readily deprotected to give an isopolar phosphonate analogue of D-fructose 1-phosphate.

The product from the acid catalysed reaction, however, was dependant on the nucleophile used. The reaction with trimethylsilyl azide yielded an anomeric azide, whereas reaction with diethyl trimethylsilyl phosphite afforded a 1′-diethyl phosphonate benzylidene due to neighbouring group participation.

ABBREVIATIONS

$[\alpha]_D$	specific optical rotation
Ac	acetyl
Ad	adamantyl
ADP	adenosine monophosphate
AIBN	azobisisobutyronitrile
AMP	adenosine monophosphate
aq.	aqueous
Ar	aryl
ATP	adenosine triphosphate
b.p.	boiling point
Bn	benzyl
Bz	benzoyl
CI	chemical ionisation
Co-A	co-enzyme A
conc.	concentrated
Cp	cyclopentadiene
CMP	cytidine monophosphate
CTP	cytidine triphosphate
DAHP	3-deoxy-D-arabino-heptulosonic acid 7-phosphate
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DCC	N,N-dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DHQ	dehydroquinate
DME	dimethoxyethane
DMF	N,N-dimethylformamide
DMSO	dimethylsulphoxide

EI	electron impact
FAB	fast atom bombardment
FBPase	fructose 1,6-bisphosphatase
h	hour
hv	irradiation
HMPA	hexamethylphosphoramide
IDCP	iodonium *sym*-dicollidine perchlorate
Im	imidazole
ir	infra-red
J	coupling constant
KDO	potassium 3-deoxy-D-manno-2-octulosonoate
LDA	lithium diisopropylamide
M	molar
min	minutes
m.p.	melting point
Ms	methanesulphonyl
N	normal
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NBS	N-bromosuccinimide
NIS	N-iodosuccinimide
nmr	nuclear magnetic resonance
n.O.e	nuclear Overhauser effect
Nu	nucleophile
o	ortho
p	para
PCC	pyridinium chlorochromate
PFK	phosphofructokinase

ppm	parts per million
Py	pyridine
R_F	retention factor
rt	room temperature
TBDPS	*t*-butyldiphenylsilyl
TBS	*t*-butyldimethylsilyl
Tf	trifluoromethanesulphonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
t.l.c.	thin layer chromatography
TMS	trimethylsilyl
Tr	triphenylmethyl
Ts	*p*-toluenesulphonyl

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PHOSPHONATES AS ANALOGUES OF NATURAL PHOSPHATES

During the past 25 years there has developed significant interest in the preparation and investigation of phosphonic acids, and their derivatives which might be considered to be analogues of naturally occurring phosphates. This interest was generated by the recognition that phosphonic acids used as analogues of naturally occurring phosphates, possessed intriguing possibilities for metabolic regulation or perturbation[1].

Since the carbon-phosphorus bond of phosphonic acids is incapable of being hydrolysed by 'ordinary enzymes' involved in phosphate cleavage, several mechanistic possibilities exist for metabolic regulation by these compounds.

For example, if a natural substrate acts as a metabolite or metabolic regulator of enzyme reactions due to a site other than the phosphate linkage, then, since the phosphonic acid is stable to enzymatic hydrolysis, the lifetime of the phosphonic acid and, hence, the integrated activity of the metabolite or regulator will increase.

Also, as a substitute for a natural metabolite, a phosphonic acid may be able to inhibit the regular metabolism of an organism by non-participation in a normal phosphate ester cleavage.

Because of the essential role played by carbohydrate phosphates in metabolic regulation, the synthesis of phosphonate analogues of common carbohydrate phosphates is of particular interest.

COMPARISON OF THE PHYSICAL PROPERTIES OF PHOSPHATES AND PHOSPHONIC ACIDS

Several changes in properties must be considered on substituting a phosphonic acid for a phosphate, aside from the stability of the phosphonic acids to enzymatic hydrolysis by normal routes[2].

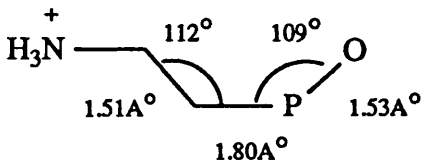
Oxygen and carbon have substantially different electronegativities (3.5 and 2.8 respectively) which results in an altered electron distribution between phosphates and phosphonates. Thus, introduction of the electron donating alkyl group decreases the acidity of the phosphorus containing acid function.

In comparison of a phosphonic acid with a phosphate, it is the second pKa which is of interest, as for either system the first represents a relatively strong acid. Croft and Kosolapoff[3] have measured the second pKa's for a series of phosphonic acids and found them to be in the range 7.7-8.2 when a primary alkyl group was attached to phosphorus. This is to be compared with the second pKa value of ca. 7.0 for the corresponding mono alkyl phosphates[4]. This could result in the existence of a different state of dissociation for the analogue compared to the natural compound at a particular (physiological) acidity associated with a biological system.

A second factor of change is that of physical size and shape. Simply replacing the phosphate ($-OPO_3H_2$) with a phosphonic acid ($-PO_3H_2$) group, obviously, contracts the overall size. More specifically, the distances between the phosphoryl oxygen and the other possible points of interaction on the molecule, for example hydroxyl groups on a carbohydrate ring, are significantly changed.

For this reason Engel in his excellent review[1] suggested that for some purposes 'isosteric' phosphonic acids, in which the O atom of the phosphate linkage has been replaced by a CH_2 group, may be considered as 'better' analogues.

X-ray diffraction analysis shows that the substitution of methylene for oxygen causes little steric deviation of the analogue from the prototype. This is illustrated by comparison of the crystallographic structures of 2-aminoethyl phosphonic acid[5] (1) and 2-aminoethyl phosphate[6] (2).



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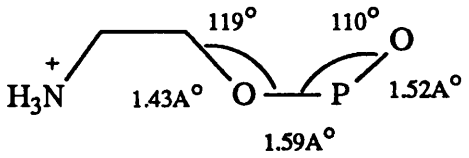

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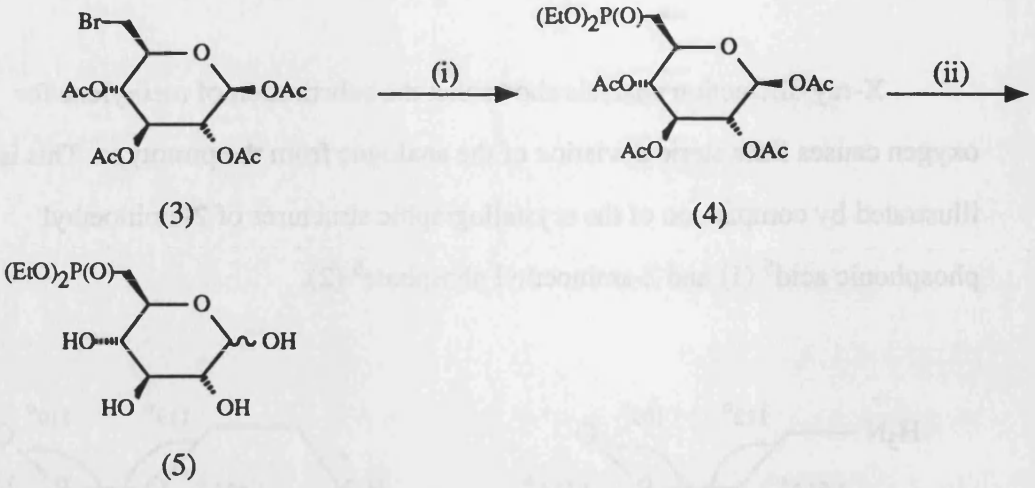
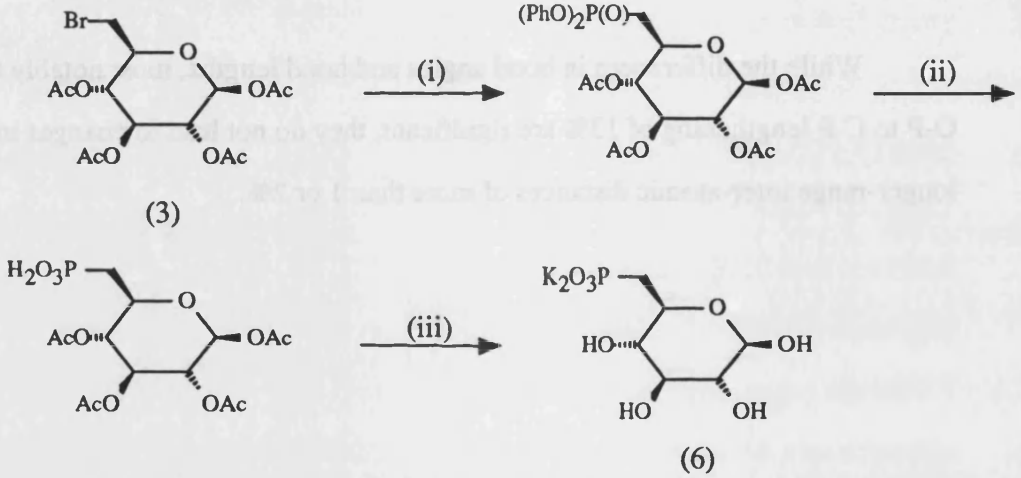
While the differences in bond angles and bond lengths, most notably the O-P to C-P lengthening of 13% are significant, they do not lead to changes in longer-range inter-atomic distances of more than 1 or 2%.

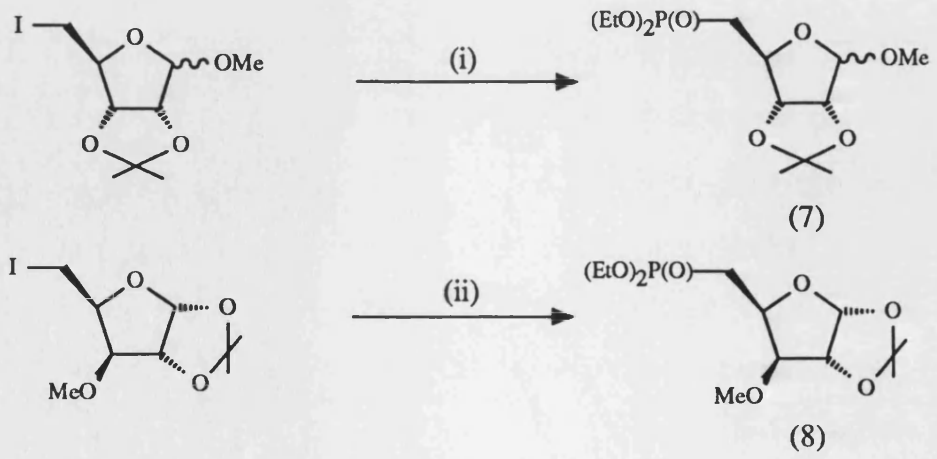


Scheme 1. *Reagents and conditions:* (i) $P(OEt)_3$, reflux, 6h (88%); (ii) 0.65N HBr, 95°, 3h



Scheme 2. *Reagents and conditions:* (i) $(PhO)_2P(OEt)$, 170°C, 62h (52%);

(ii) H_2, PtO_2, abs EtOH (76%); (iii) KOMe, MeOH (50%)



Scheme 3. *Reagents and conditions:* (i) $(EtO)_3P$, reflux, 10h (31%);

(ii) $(EtO)_3P$, reflux, 7h (90%).

SYNTHESIS OF CARBOHYDRATE PHOSPHONATES

Several approaches have been made toward the preparation of phosphonic acid analogues related to common carbohydrates. Griffin and Burger early reported the preparation of 6-deoxy-D-glucose 6-phosphonic acid (6)[7], an analogue of D-glucose 6-phosphate.

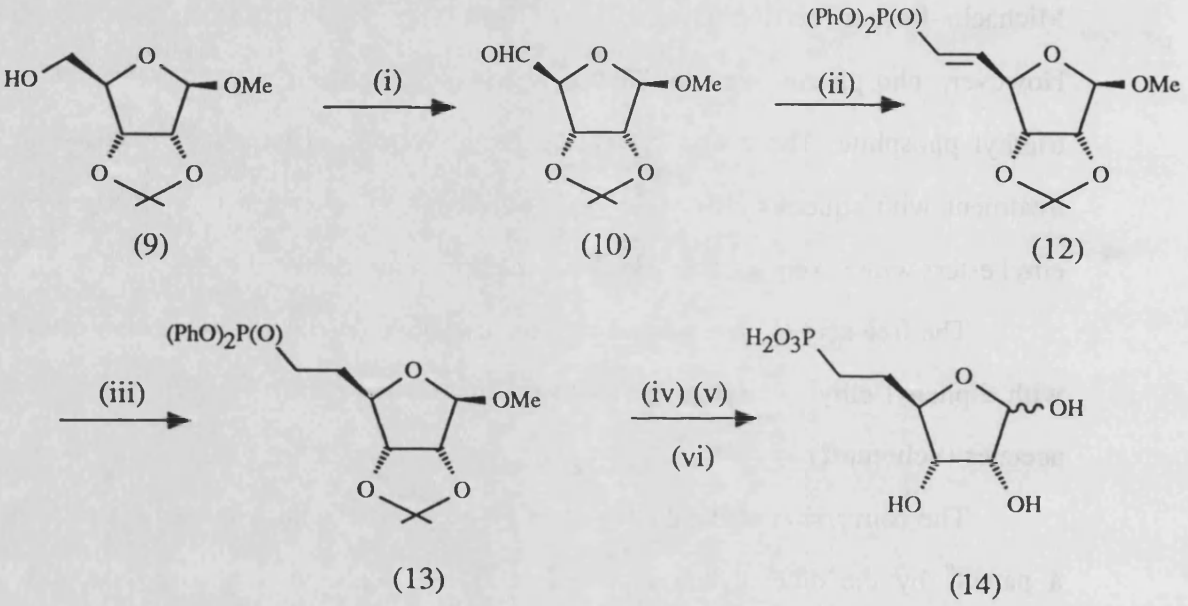
The protected 6-deoxy-6-bromo-D-glucose (3) proved inert to Michaelis-Becker reaction with sodium dialkylphosphites in hydrocarbon solvents. However, phosphorus was introduced via a Michaelis-Arbuzov reaction with triethyl phosphite. The acetyl protecting groups could be removed from (4) on treatment with aqueous HBr yielding (5) although all attempts to hydrolyse the ethyl esters with strong acids resulted in decomposition (**Scheme 1**).

The free acid (6) was ultimately prepared *via* a Michaelis-Arbuzov reaction with diphenyl ethyl phosphite followed by hydrogenolysis and hydrolysis of the acetates (**Scheme 2**).

The conversion of the diethyl ester (4) to the free acid was later claimed in a patent[8] by the didealkylation procedure using alkali halides. This procedure involves heating the phosphonate diester to 150°C with sodium iodide in DMF followed by treatment with aqueous acetic acid.

The introduction of the phosphonic diester function *via* the Michaelis-Arbuzov reaction with triethyl phosphite has also been reported by Parikh *et al.*[9] and Whistler and Wang[10] for the generation of non-isosteric phosphonate (diester) analogues related to D-ribose (7) and D-xylose (8) terminal phosphates (**Scheme 3**). Again, free phosphonic acids were not obtained from the diethyl esters.

A highly convenient route to isosteric analogues of terminal phosphates was developed by Jones *et al.*[11] involving the use of a stabilized Wittig reagent. Using suitably protected carbohydrates with a 5- or 6-hydroxyl free (for pentoses

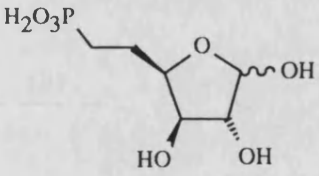


Scheme 4. *Reagents and conditions* (i) DMSO, DCC (ii) $Ph_3P{=}CH(O)P(OPh)_2$ (11); (iii) H_2, $Pd/BaSO_4$; (iv) NaOH, BnOH; (v) H_2, $Pd/BaSO_4$; (vi) 90% aq TFA.

and hexoses, respectively). Thus, the appropriately protected D-ribose derivative (9) was oxidised to the aldehyde (10) using the previously developed dimethyl sulphoxide-dicyclohexyl carbodiimide technique[12]. Treatment of (10) with the stabilized ylid $Ph_3P{=}CHP(O)(OPh)_2$ (11)[13,14] yielded the vinylphosphonate (12), exclusively as the trans isomer.

The vinylphosphonate was hydrogenated to give the isosteric phosphonate diphenyl ester (13) which on transesterification and deprotection gave the isosteric phosphonate analogue 5-deoxy-5-dihydroxyl phosphinylmethyl-D-ribose (14) of D-ribose 5-phosphate (**Scheme 4**).

This approach was also utilised[11] for the preparation of the corresponding isosteric phosphonic acids related to D-xylose 5-phosphate (15), D-lyxose 5-phosphate (16), D-arabinose 5-phosphate (17), 2-deoxy-D-ribose 5-phosphate (18), D-glucose 6-phosphate (19) and D-mannose 6-phosphate (20), although complete experimental details for each are not provided in the patent.



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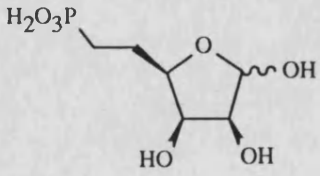

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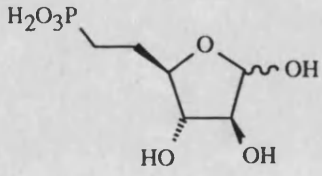

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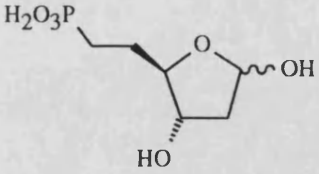

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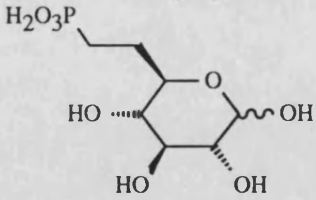

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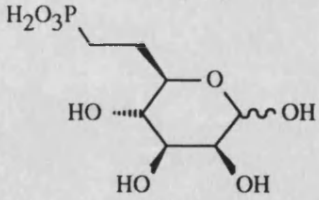
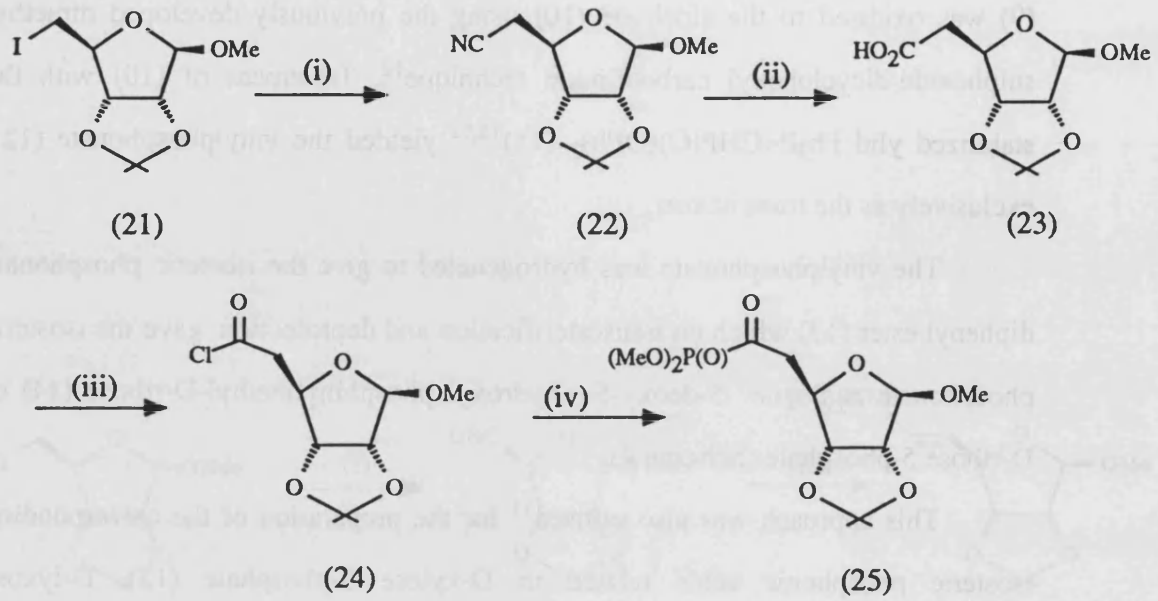
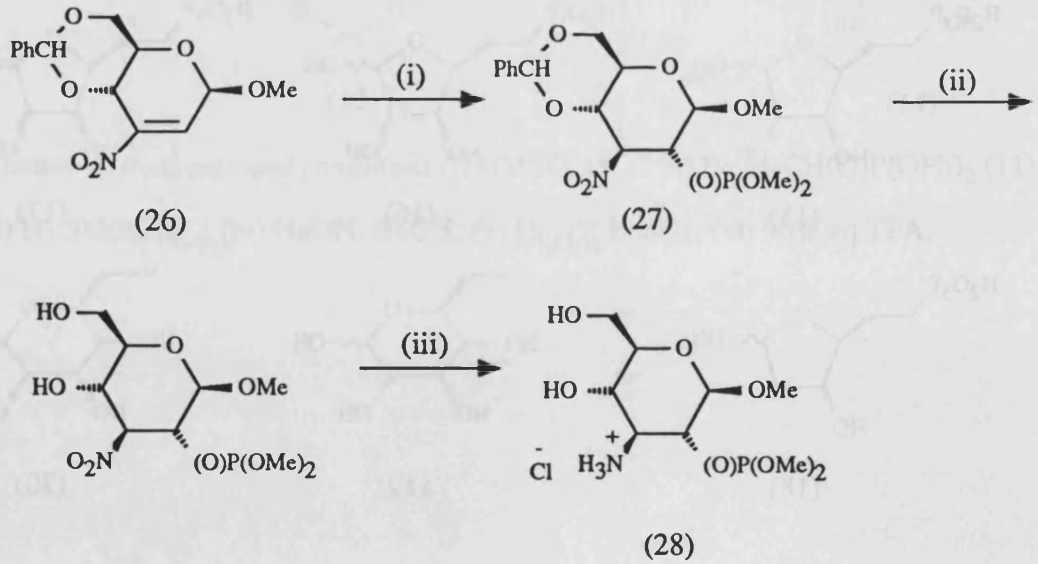


(20)

More recently the same approach has been described by Adams *et al.*[14] for the preparation of the D-glucose 6-phosphate analogue (19) and by Unger *et al.*[15] for the D-arabinose 5-phosphate analogue (17). The D-glucose 6-phosphate analogue (19) was dehydrogenated by $NADP^+$ in the presence of glucose



Scheme 5. *Reagents and conditions:* (i) NaCN, DMF, 50°C, 4h (52%); (ii) 10% aq. KOH, MeOH, reflux, 25min (55%); (iii) $SOCl_2$, reflux, 30 min; (iv) $(MeO)_3P$, 3h, rt.



Scheme 6. *Reagents and conditions:* (i) $(MeO)_2P(O)H$, Et_3N(cat), rt, 2h (69%); (ii) aq. HCl, rt, 24h (56%); (iii) H_2, PtO_2, aq. HCl (98%).

6-phosphate dehydrogenase[14], showing Michaelis-Menten kinetics. However, the affinity of (19) for the enzyme was significantly weaker than that of glucose 6-phosphate and the K_M values of (19) were 4-5 fold higher than those of the natural substrate.

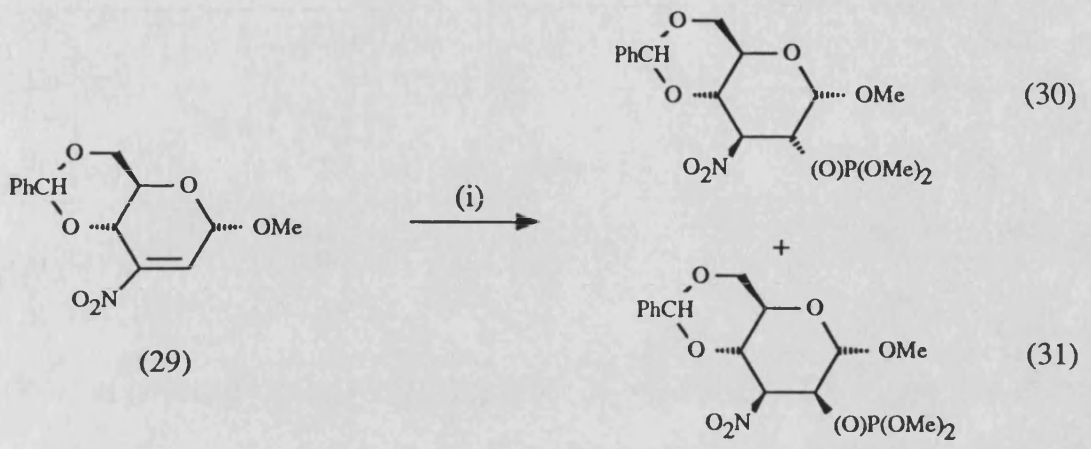
Hampton *et al.* have reported[16] the synthesis of an isosteric α-keto phosphonate analogue of D-ribose 5-phosphate. Treatment of 5-deoxy-5-iodo D-ribofuranoside (21) with sodium cyanide gave the 5-cyano-5-deoxy D-ribofuranoside (22). Alkaline hydrolysis afforded the carboxylic acid (23) which was transformed to the acyl chloride (24) on treatment with thionyl chloride. The acid chloride was found to react rapidly with trimethyl phosphite to give the α-keto phosphonate (25) in good yield (**Scheme 5**).

However, the α-keto phosphonate (25) was unstable even under anhydrous conditions and was readily hydrolysed to the acid (23) and dimethyl phosphite. No attempts were made to transform (25) to the phosphonic acid.

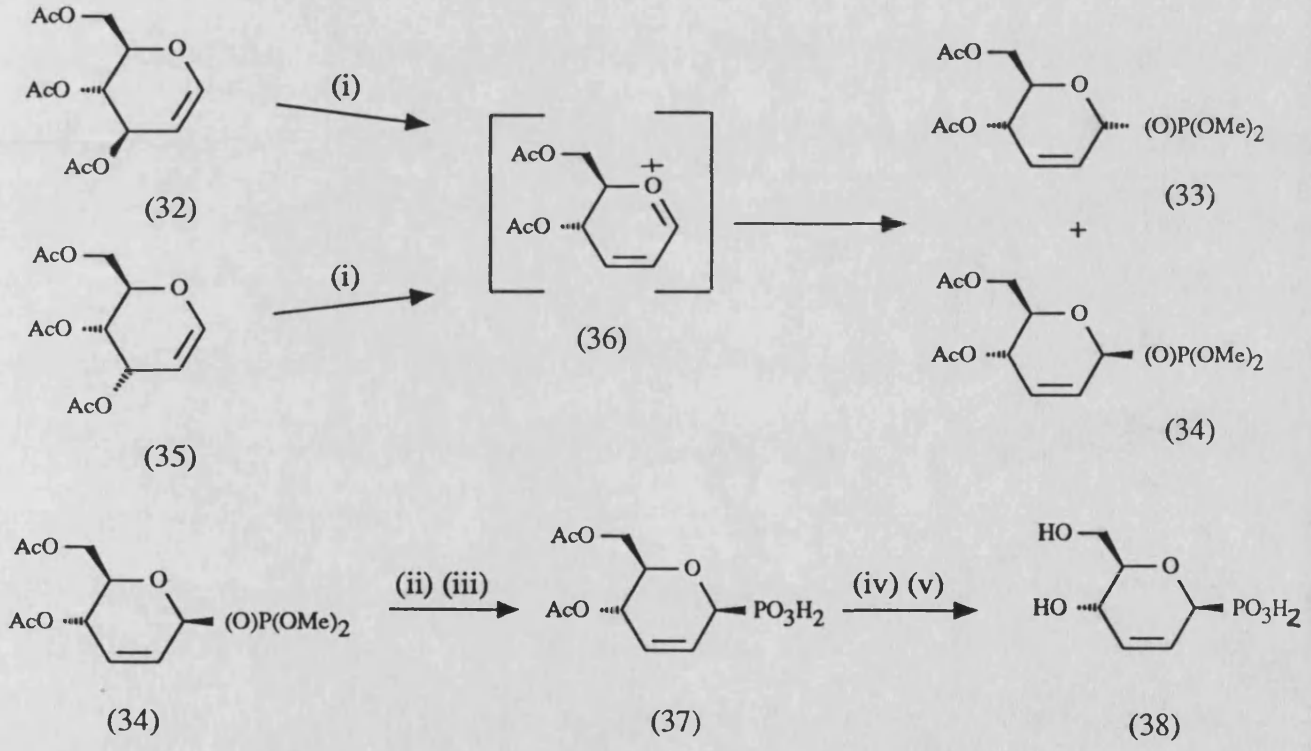
In a series of papers Paulsen *et al.* achieved phosphorylation of a variety of carbohydrates by addition to double bonds. The β-D-erythro-hex-2-ene-pyranoside derivative (26) containing an activated double bond reacted with dialkyl phosphites in the presence of a catalytic amount of triethylamine to yield preferentially the 3-nitro-2-phosphono-gluco derivative (27)[17] (**Scheme 6**).

Hydrolysis of (27) and subsequent hydrogenation afforded the 3-amino-2-phosphono compound (28). The corresponding addition to the α-glycoside (29) gave the gluco and manno products (30) and (31) respectively, in approximately equal amounts (**Scheme 7**).

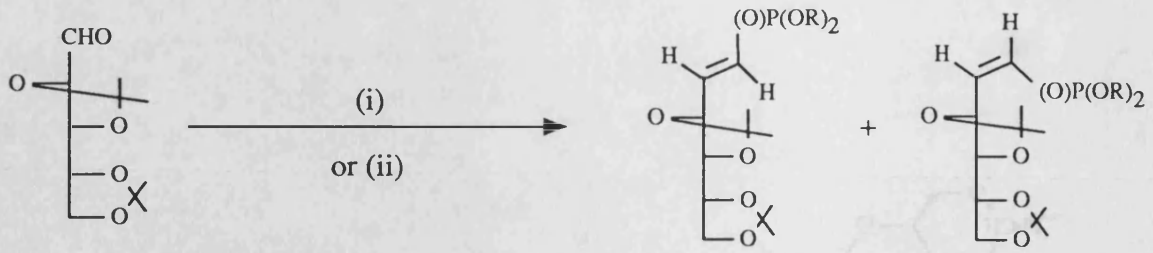
The synthesis of glycopyranosyl phosphonates was achieved by Lewis acid catalysed addition of dimethyl phosphites to glycals with allylic shift[18]. Thus, reaction of 3,4,6-tri-O-acetyl-1,2-dideoxy-D-arabino-hex-1- enopyranose (32) in dimethyl phosphite catalysed by boron trifluoride affords the α- and β-D-erythro-hex-2-enopyranoside phosphonates (33) and (34) in a 1:2 ratio.



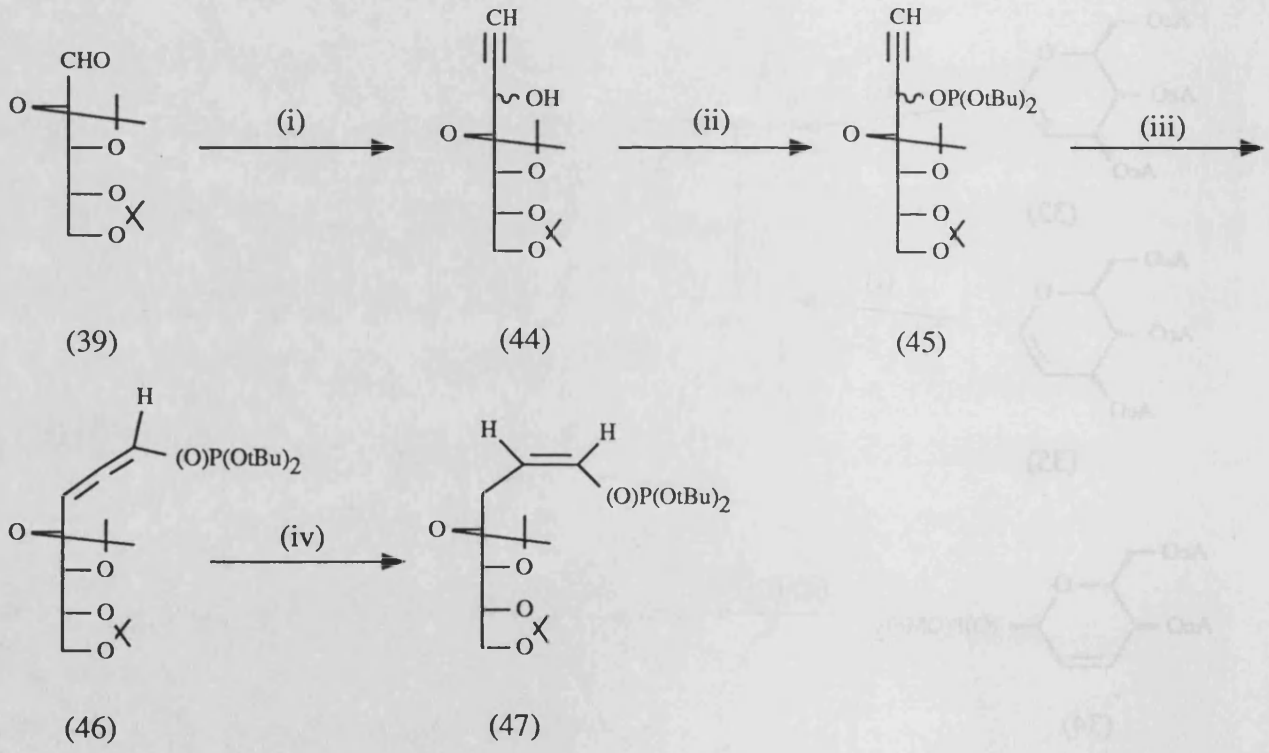
Scheme 7. *Reagents and conditions:* (i) $(MeO)_2P(O)H$, Et_3N(cat), rt, 2h (65%).



Scheme 8. *Reagents and conditions:* (i) $P(OMe)_3$, $BF_3.Et_2O$, 60°C, 2h; (ii) TMSCl, reflux; (iii) H_2O; (iv) NaOMe, MeOH; (v) Dowex (H^+), MeOH (80% from 36).



Scheme 9. *Reagents and conditions:* (i) $NaCH((O)P(OEt)_2)_2$, DME, rt, 1h (87%); (ii) $LiCH(SiMe_3)(O)P(OMe)_2$, THF, -5°, 3h



Scheme 10. *Reagents and conditions:* (i) $BrMgC{\equiv}CH$; (ii) $Cl(O)P(^tOBu)_2$, PhH, Et_3N, 5 - 10°, 2h; (iii) rt; (iv) H_2, $Pd/BaSO_4$, PhH (53%).

The reaction of the corresponding ribo compound (35) affords the same anomeric distribution of (33) and (34). This finding supports a S_N1' reaction with rearrangement (**Scheme 8**).

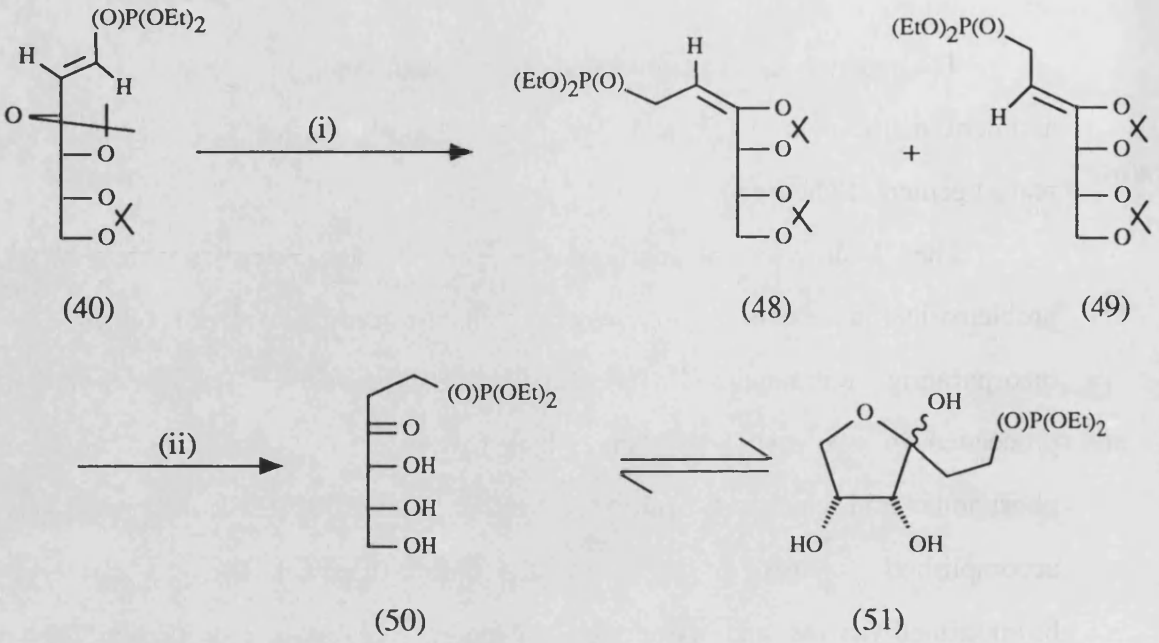
The hydrolysis of the dialkyl phosphonate ester, which proved problematical in the earlier syntheses of phosphonic acids, was readily achieved by incorporating Rabinowitz's[19] use of halotrialkylsilanes. The dealkylation proceeded in two steps, with the initial formation of a disilyl ester of the phosphonic acid which was hydrolysed by dissolution in water. The cleavage was accomplished using refluxing chlorotrimethylsilane, however, bromotrimethylsilane and iodotrimethylsilane have subsequently proved to be more efficient.

Basic hydrolysis of (37) followed by treatment with an acidic ion-exchange resin afforded the fully deprotected phosphonic acid (38).

The Horner-Emmons reaction of 2,3:4,5-di-O-isopropylidene-D- arabinose (39) with sodium methanebis(diethyl phosphonate) afforded exclusively the trans olefinic phosphonate (40). Whereas, Peterson-Carey reaction of (39) with lithium trimethylsilylmethane (dimethyl phosphonate) gave approximately a 1:2 ratio of the trans- and cis-olefinic phosphonates (41) and (43) respectively[20] (**Scheme 9**).

Cis-olefinic phosphonates were also prepared from Mark rearrangement[21] of 1-alkyne-3-phosphites followed by hydrogenation[20]. Thus, the aldehydo sugar (39) gave on Grignard reaction with ethynyl magnesium bromide a diastereomeric mixture of 1-alkyne-3-hydroxy sugars (44). The propargyl alcohols reacted with di-^{t}butyl chlorophosphite to afford the trivalent phosphorus esters (45) which rearranged spontaneously at room temperature to yield the allenic phosphonate (46). The allene could be hydrogenated to give the 3-deoxy-cis-olefinic phosphonate (47) (**Scheme 10**).

The trans-olefinic phosphonate could be rearranged on treatment with sodium ethoxide or piperidine to the corresponding allyl phosphonates. These were

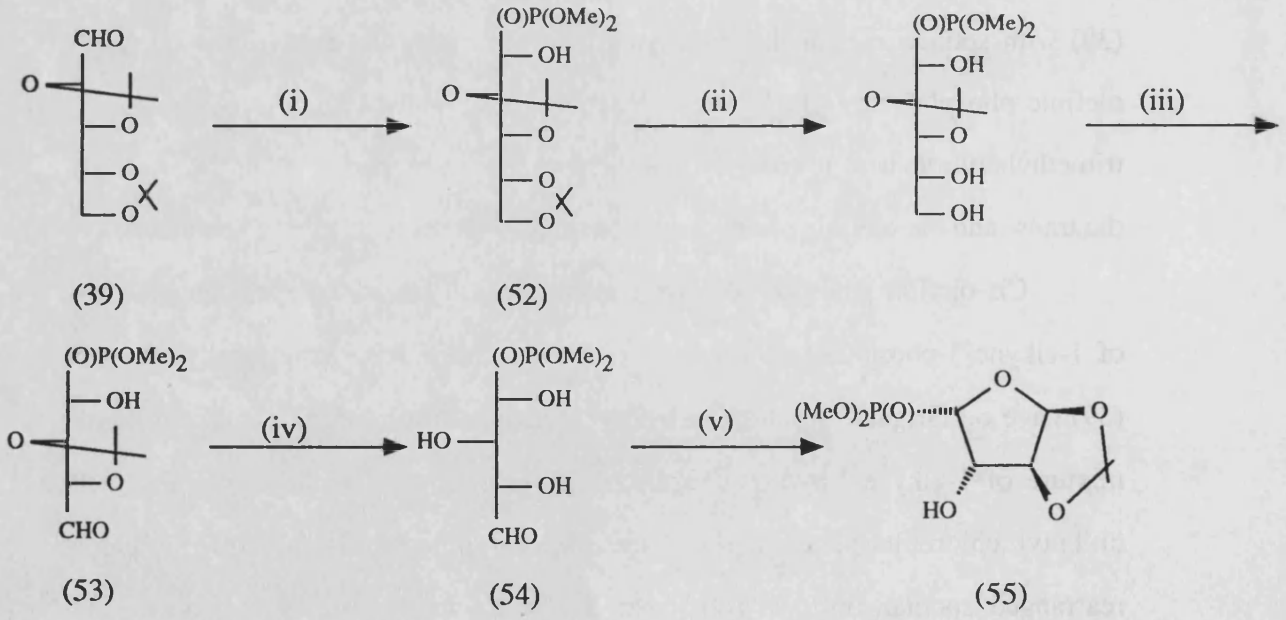


(40) (48) (49)

(50) (51)

Scheme 11. *Reagents and conditions:* (i) NaOEt, EtOH, reflux, 4h (90%);

(ii) 25% AcOH, 50°, 3h, (94%).



(39) (52)

(53) (54) (55)

Scheme 12. *Reagents and conditions:* (i) $(MeO)_2P(O)H$, NaOMe (86%); (ii) 70% AcOH, rt, 17h (73%); (iii) $NaIO_4$, H_2O, EtOH, rt, 30min (88%); (iv) 50% AcOH, reflux, 1h (97%);

(v) H_2SO_4, acetone (91%).

obtained as a cis and trans mixture (48) and (49) respectively with the trans isomer predominating[22].

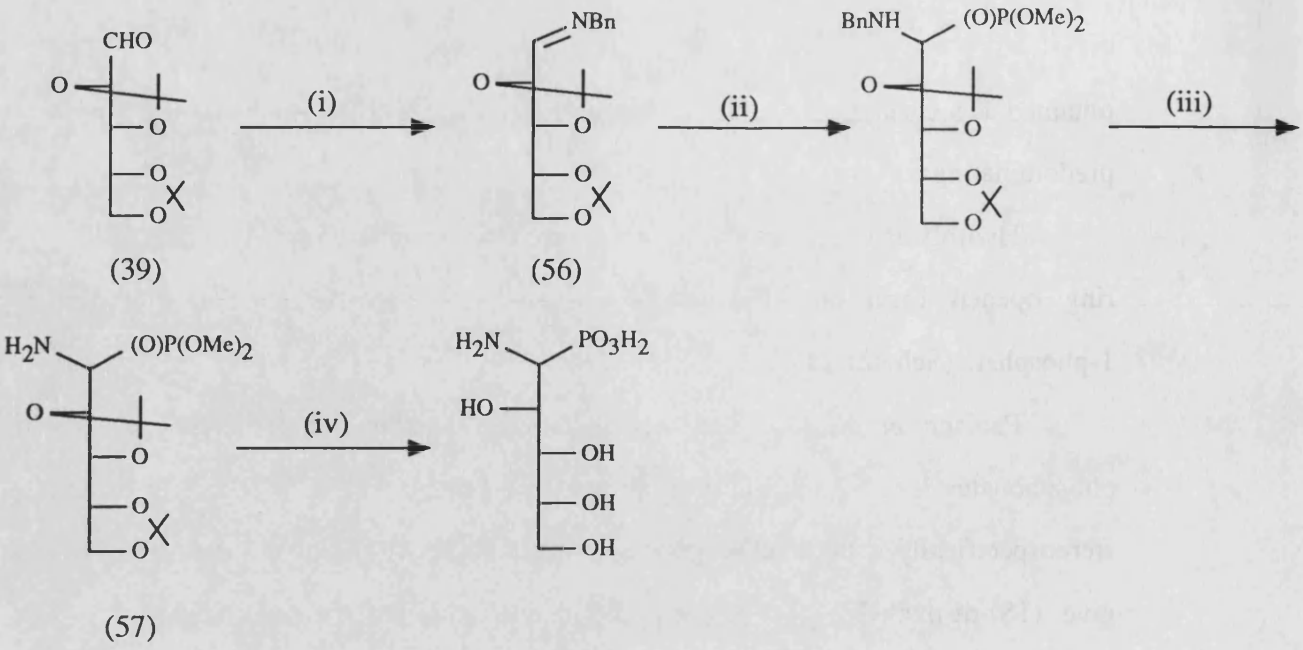
Hydrolysis of the cis and trans mixture affords the ketose (50) which is the ring opened form of the isosteric phosphonate analogue (51) of ribulose 1-phosphate (**Scheme 11**).

Paulsen *et al.* also used the Abramov reaction for the synthesis of phosphonates[23]. 2,3:4,5-Di-O-isopropylidine-D-arabinose (39) reacted stereospecifically with dimethyl phosphite in the presence of sodium methoxide to give (1S)-di-methyl-2,3:4,5-di-O-isopropylidene-D-arabitol-1- phosphonate (52). Partial deprotection on treatment with acetic acid followed by oxidation with sodium periodate affords the aldehyde (53). Deprotection gave (4S)-dimethyl-D-threofuranose-4-phosphonate (54) isolated as the crystalline 1,2-O-isopropylidene (55) (**Scheme 12**).

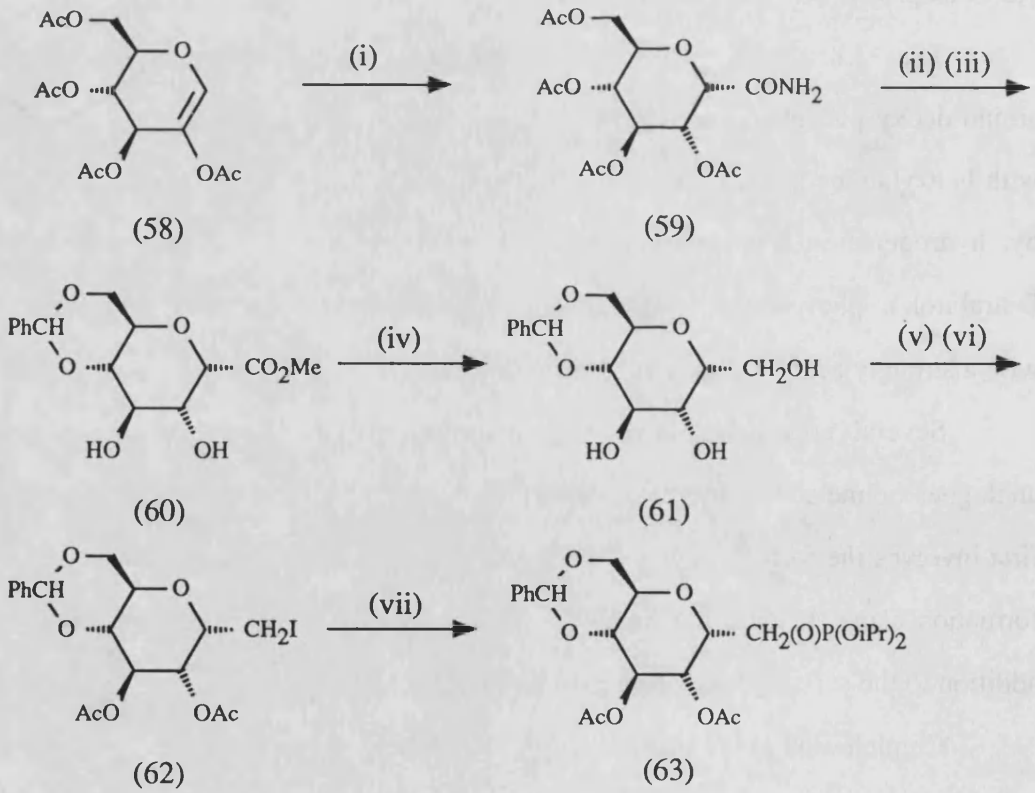
The Abramov reaction was also modified for the synthesis of amino-deoxy-phosphono derivatives[24]. Condensation of the arabino aldehyde (39) with benzylamine affords the imine (56). Addition of dimethyl phosphite followed by hydrogenation gave dimethyl 1-amino-1-deoxy-2,3:4,5-di-O-isopropylidene D-arabitol-1 -phosphonate (57). Complete deprotection was affected on treatment with a strongly acidic ion exchange resin (**Scheme 13**).

Several approaches have been made toward the synthesis of isosteric analogues bound to the anomeric centre. Two strategies have been employed, the first involves the formation of a C-glycoside followed by carbon-phosphorus bond formation using standard reactions. The second and the more convenient involves addition to the carbohydrate of a methylene phosphonate unit.

Chmielewski *et al.* utilised the first strategy for the synthesis of an isosteric analogue of α-D-glucose-1-phosphate[25]. Carbamoylation of 1,5-anhydro-D-arabino-hex-1-enitol (58) with formamide yielded stereospecifically the α-amide (59). Methanolysis with 7% HCl in methanol at



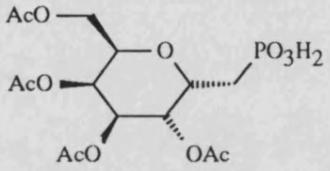
Scheme 13. *Reagents and conditions:* (i) $BnNH_2$, 60°, 1h; (ii) $(MeO)_2P(O)H$, 60°, 20h, (72%); (iii) H_2, Pd/C, MeOH (92%); (iv) Levatit S100 (H^+), H_2O, 90°, 20h (93%).



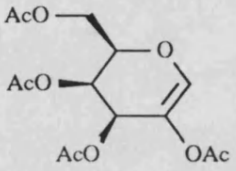
Scheme 14. *Reagents and conditions:* (i) $HCONH_2$, acetone, 72h (50%); (ii) 7% HCl, MeOH, rt, 24h; (iii) $ZnCl_2$, PhCHO, rt, 48h (64%); (iv) $NaBH_4$, THF, H_2O; (v) NIS, Ph_3P, DMF, 50°, 24h; (vi) Ac_2O, Py (30%); (vii) $(^iPrO)_3P$, 180°, 24h (43%).

reflux followed by reprotection gave the ester (60). The ester was reduced with sodium borohydride to the alcohol (61) which on treatment with N-iodosuccinimide and triphenylphosphine afforded the primary iodide (62). Protection as the diacetate followed by Michaelis-Arbuzov reaction with refluxing triisopropyl phosphite gave the isosteric analogue (63) of α-D-glucose-1-phosphate, although the free phosphonic acid was not subsequently obtained (**Scheme 14**).

The isosteric analogue (64) of α-D-galactose-1-phosphate was similarly prepared from 1,5-anhydro-D-lyxo-hex-1-enitol (65)[25].

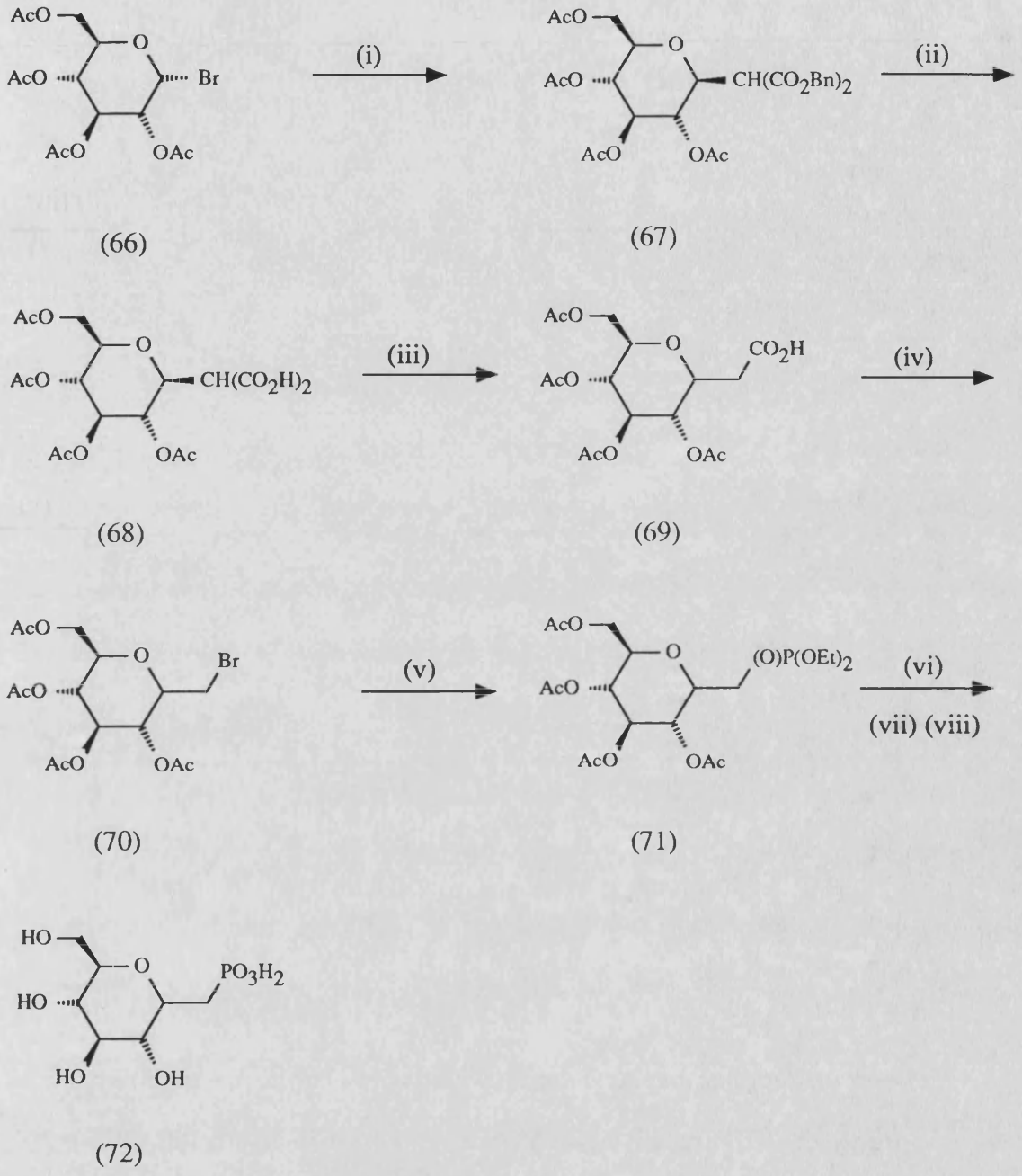


(64)

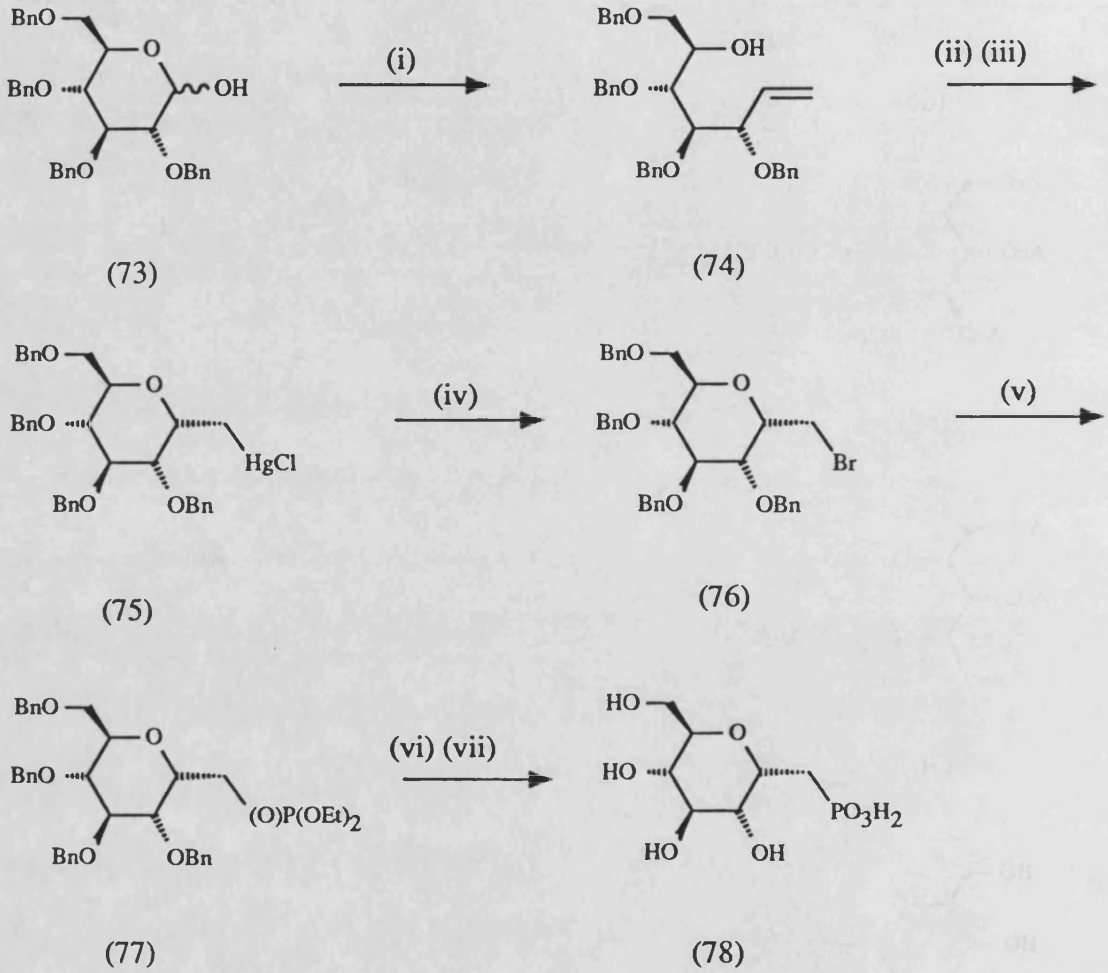


(65)

Russo *et al.* initially attempted the introduction of the methylene phosphonic group to a carbohydrate moiety by reaction of the α-D-glucopyranosyl bromide (66) with $LiCH_2P(O)(OMe)_2$ in the presence of HMPA to enhance the softness of the nucleophile[26]. However, only the elimination product (58) was obtained[27]. Again the C-glycosyl intermediate had to be prepared first linking the the P atom afterwards. The 2,6-anhydro-1-bromo-1-deoxy-D-glycero D-gluco-hepitol (70) was synthesised as reported by Hanessian *et al.*[28] in 35% yield from the tetra-O-acetyl-α-D-glucopyranosyl bromide (66). Thus, (66) condensed smoothly with sodium dibenzyl malonate to give the C-glycosyl malonate (67) exclusively with the β-configuration. Hydrogenation over 20% palladium on charcoal gave the malonic acid (68) which was decarboxylated quantitatively in refluxing acetic acid yielding the monoacid (69). Modified Hunsdiecker reaction[29] afforded the bromide (70).



Scheme 15. *Reagents and conditions:* (i) $CH_2(CO_2Bn)_2$, NaH, DME, rt, 48h, (80%); (ii) H_2, Pd/C, EtOH (51%); (iii) AcOH, reflux, 3h (100%); (iv) HgO, Br_2, CCl_4, reflux, 3h (87%); (v) $(EtO)_3P$, reflux, 6h (90%); (vi) TMSI, CCl_4, 0°, 30min; (vii) H_2O; (viii) Dowex 2 (OH^-), 24h (60%).



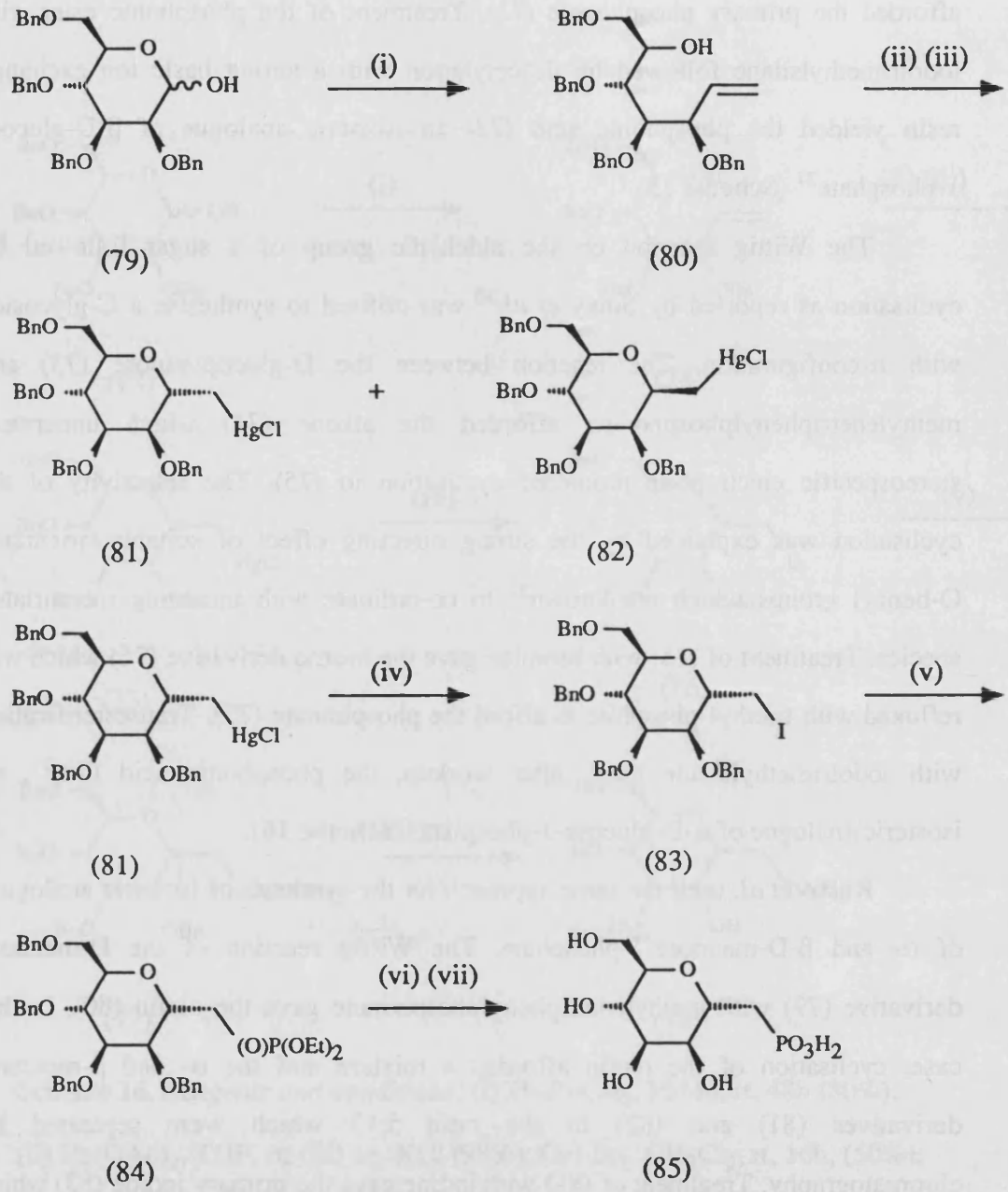
Scheme 16. *Reagents and conditions:* (i) $Ph_3P{=}CH_2$, PhMe, rt, 48h (80%); (ii) $Hg(OAc)_2$, THF, rt; (iii) aq. KCl (98%); (iv) Br_2, CH_2Cl_2, rt, 16h, (50%); (v) $(EtO)_3P$, reflux, 7h (60%); (vi) TMSI, CCl_4, 0°, 30min; (vii) H_2O.

Michaelis-Arbuzov reaction of (70) with refluxing triethyl phosphite afforded the primary phosphonate (71). Treatment of the phosphonic ester with iodotrimethylsilane followed by deacetylation with a strong basic ion exchange resin yielded the phosphonic acid (72) an isosteric analogue of β-D-glucose 1-phosphate[27] (**Scheme 15**).

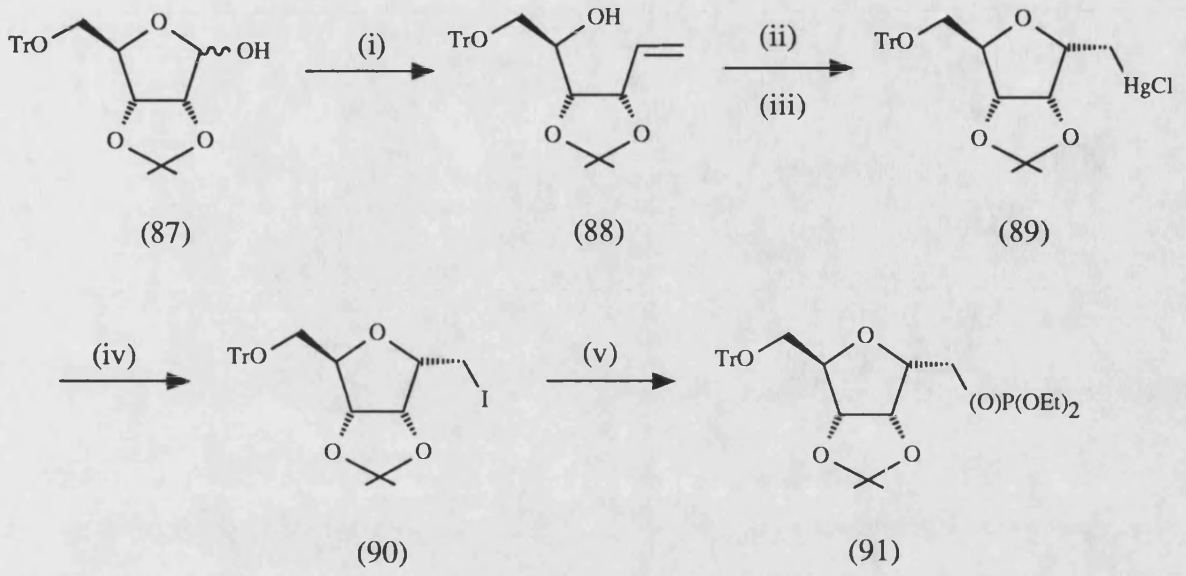
The Wittig reaction on the aldehydic group of a sugar followed by cyclisation as reported by Sinay *et al.*[30] was utilised to synthesise a C-glycoside with α-configuration. The reaction between the D-glucopyranose (73) and methylenetriphenylphosphorane afforded the alkene (74) which underwent stereospecific electrophile promoted cyclisation to (75). The selectivity of the cyclisation was explained by the strong directing effect of suitably orientated O-benzyl groups which are known[31] to co-ordinate with incoming mercuriated species. Treatment of (75) with bromine gave the bromo derivative (76) which was refluxed with triethyl phosphite to afford the phosphonate (77). Transesterification with iodotrimethylsilane gave, after workup, the phosphonic acid (78)[27], an isosteric analogue of α-D-glucose-1-phosphate (**Scheme 16**).

Russo *et al.* used the same approach for the synthesis of isosteric analogues of α- and β-D-mannose-1-phosphate. The Wittig reaction of the D-mannose derivative (79) with methylenetriphenylphosphorane gave the olefin (80). In this case, cyclisation of the olefin afforded a mixture and the α- and β-mercurio derivatives (81) and (82) in the ratio 5:13 which were separated by chromatography. Treatment of (81) with iodine gave the primary iodide (83) which on Michaelis-Arbuzov reaction with refluxing triethyl phosphite afforded the primary phosphonate (84). The protecting groups were removed in one step employing six equivalents of iodotrimethylsilane to give the isosteric analogue (85) of α-D-mannose-1-phosphate[32] (**Scheme 17**).

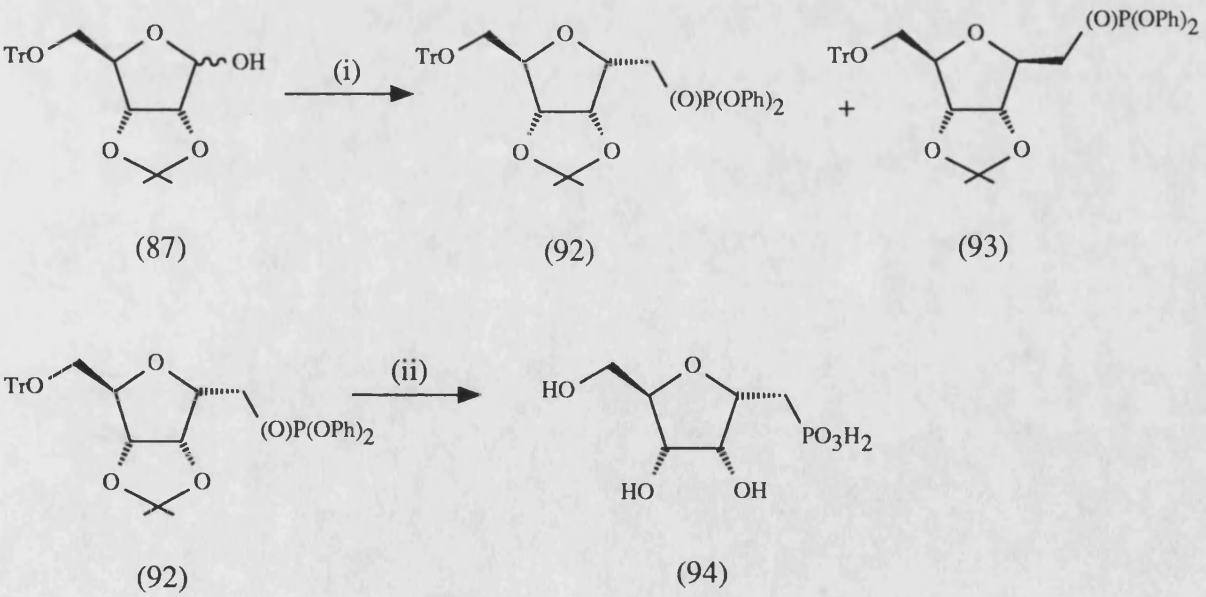
In an identical sequence of reactions the isosteric analogue (86) of β-D-mannose-1-phosphate was prepared from the mercurio compound (82)[32].



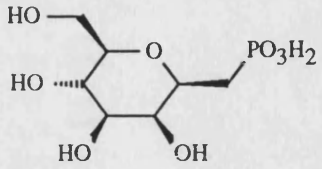
Scheme 17. *Reagents and conditions:* (i) $Ph_3P{=}CH_2$, THF, 45°, 15h (34%); (ii) $Hg(OAc)_2$, THF, rt, 24h; (iii) aq. KCl (95%); (iv) I_2, CH_2Cl_2, rt, 1h, (84%); (v) $(EtO)_3P$, reflux, 3h (84%); (vi) TMSI, CCl_4, 0°, 30min; (vii) H_2O (80%).



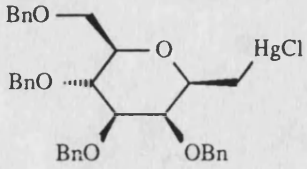
Scheme 18. *Reagents and conditions:* (i) $Ph_3P{=}CH_2$ (50%); (ii) $Hg(OAc)_2$, THF, rt, 1h; (iii) aq. KCl (82%); (iv) I_2, CCl_4 (80%); (v) $(EtO)_3P$, reflux, 4h (70%).



Scheme 19. *Reagents and conditions:* (i) $Ph_3P{=}CH(O)P(OPh)_2$ (50%); (ii) 7% HCl, AcOH, rt, 1h (55%).



(86)



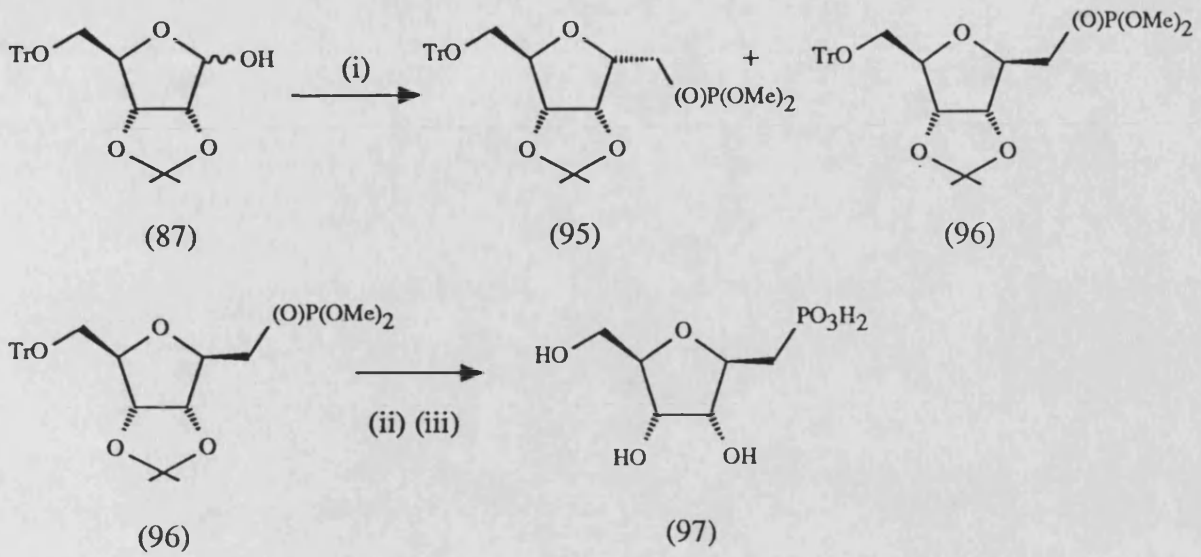
(82)

Subsequently Nicotra *et al.* extended this methodology to a furanose sugar[33]. In this case, reaction of 2,3-O-isopropylidene 5-trityl-D-ribose (87) with methylenetriphenylphosphorane afforded the alkene (88). Cyclisation again gave good stereoselection to yield the 1,2-cis-glycoside (α/β ratio 95:5). The predominant α-anomer (89) was easily isolated by chromatography. Treatment of (89) with iodine afforded the iodo derivative (90) which was submitted to Michaelis-Arbuzov reaction in refluxing triethyl phosphite to give the primary phosphonate (91) an isosteric analogue of α-D-ribose-1-phosphate (**Scheme 18**).

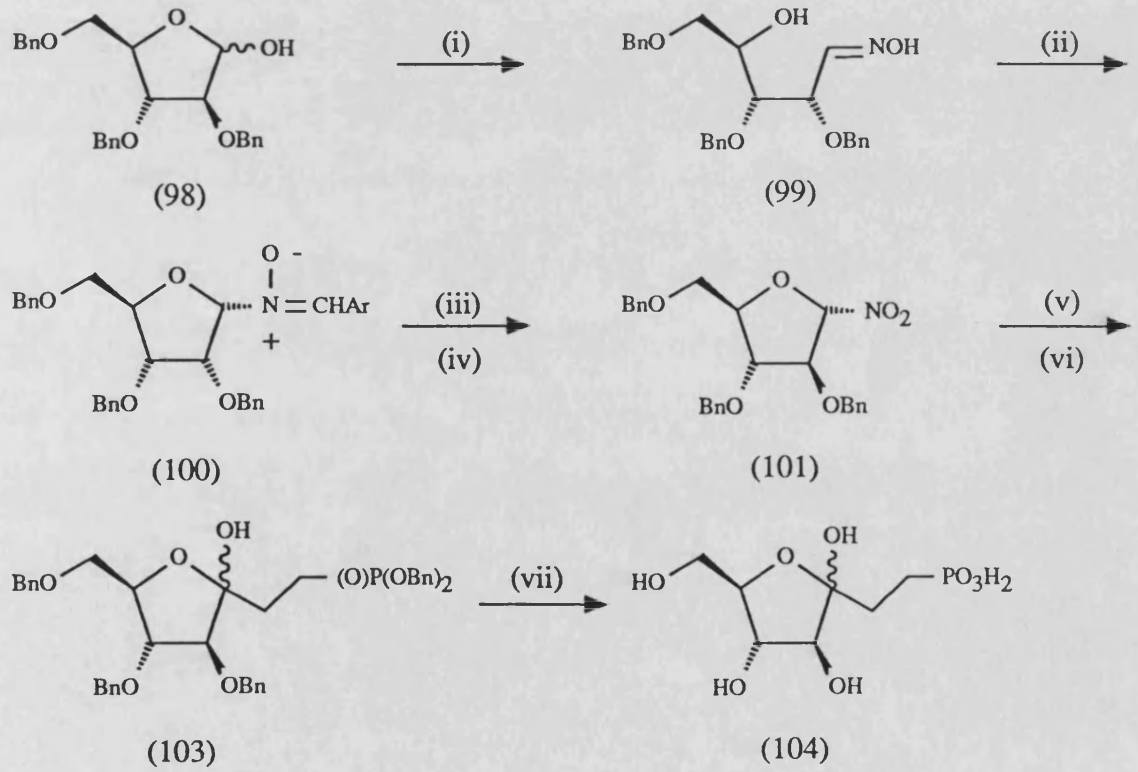
McClard was the first to report a single step approach to the synthesis of aldose sugar phosphonates substituted at the anomeric carbon[34]. The ribose derivative (87) reacted with the stabilised ylid diphenyl triphenyl phosphoranylidenemethyl phosphonate, previously utilised by Jones *et al.*[11], to yield a readily separable anomeric mixture of C-glycosides (92) and (93) (α/β ratio 1:4).

The intermediate olefin had undergone a spontaneous Michael-type addition to reclose the sugar ring at the newly formed 'anomeric' carbon. The minor α-anomer was readily hydrolysed to yield the isosteric analogue (94) of α-D-ribose-1-phosphate (**Scheme 19**).

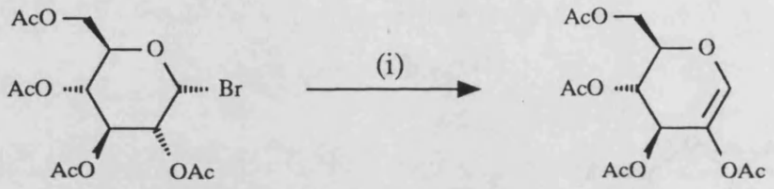
Independently, Meyer *et al.* utilised the Horner-Emmons modification of the Wittig reaction to synthesise an anomeric methylene phosphonate[35]. Reaction of the D-ribose derivative (87) with tetramethyl methylene bisphosphonate proceeded smoothly at room temperature in a biphasic mixture of methylene



Scheme 20. *Reagents and conditions:* (i) $((MeO)_2P(O))_2CH_2$, NaOH, CH_2Cl_2, rt, 18h, (61%); (ii) TMSBr, $CHCl_3$; (iii) 50% aq. TFA (60%).



Scheme 21. *Reagents and conditions:* (i) NaOMe, $H_2NOH.HCl$, MeOH, rt, 2h (100%); (ii) *p*-$NO_2C_6H_4CHO$, TsOH, Drierite, CH_2Cl_2, rt, 2h; (iii) O_3, CH_2Cl_2, -78°; (iv) $NaBH_4$, NaCl, diglyme, rt, 3h (76%); (v) CH_2=CH(O)$P(OBn)_2$ (102), Bu_4NF, THF, rt, 1h; (vi) aq. $NaHCO_3$, 60°, 24h (83%); (vii) H_2, Pd/C, dioxane, H_2O (100%).



(107) (108)

Scheme 22. *Reagents and conditions:* (i) $(EtO)_3P$, reflux (75%).

chloride and 50% aq. NaOH to give a mixture of C-glycosides (95) and (96). In this case, the anomeric mixture was enriched in the α-anomer (95) (α/β ratio 3:2). The two anomers could be separated by preparative t.l.c. at this point. Deprotection was readily accomplished by transesterification with bromotrimethylsilane followed by hydrolysis with 50% aq. TFA, to give the α-(94) and β-(97) analogues of D-ribose 1-phosphate (**Scheme 20**).

Both the pure anomers (94) and (97) were tested as inhibitors of bovine spleen purine nucleoside phosphorylase. The reversible enzymic reaction was assayed in the biosynthetic direction with D-ribose 1-phosphate (0.4mM) and guanine(0.2mM) as substrates. However, at concentrations as high as 6mM neither (94) or (97) gave any inhibition of purine nucleoside phosphorylase[37].

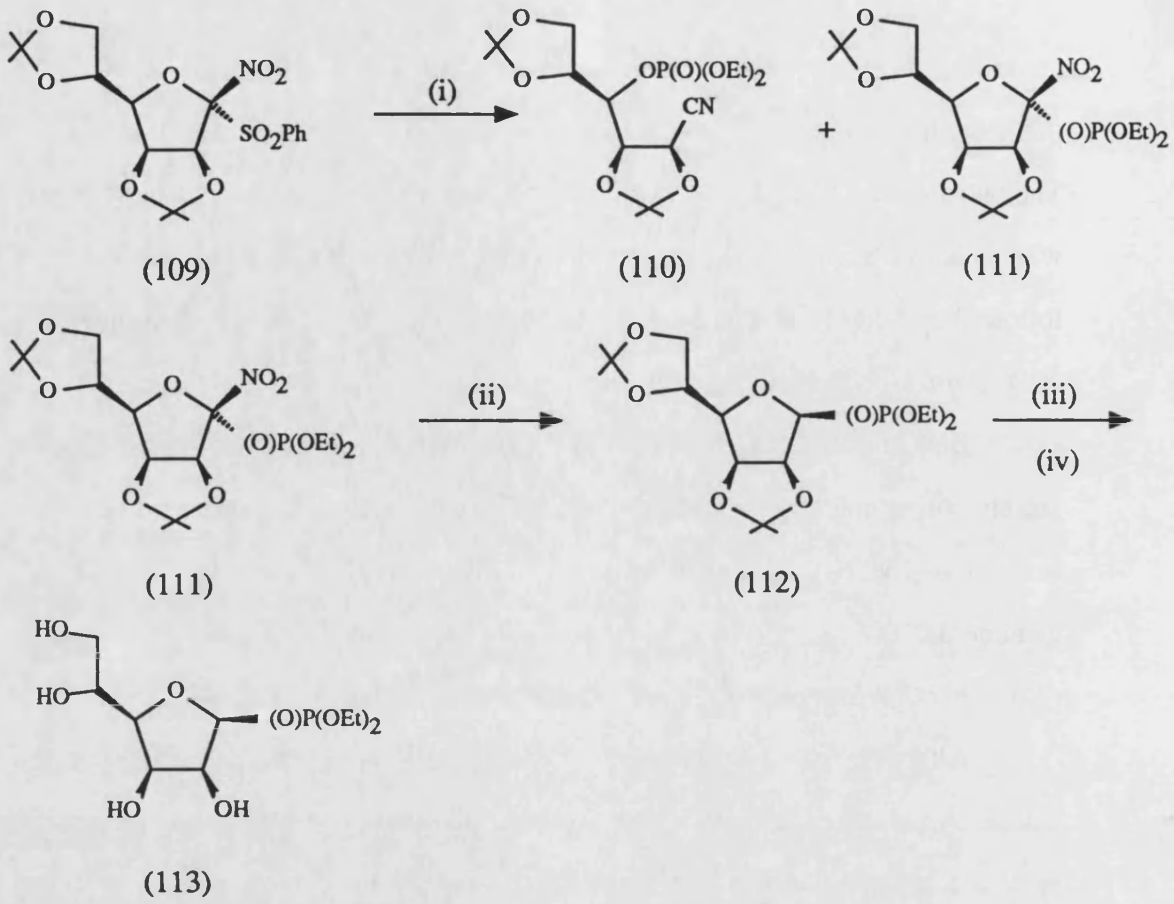
Although this methodology lends itself well to the synthesis of aldose-1-phosphates it cannot be extended to ketose-1-phosphates. A general synthesis of phosphonate analogues of ketoses was developed by Julina and Vasella[36] from readily available 1-deoxy-1-nitro-aldoses[37].

2,3,5-Tri-O-benzyl-D-arabinose (98) was converted to the nitro sugar (101) *via* the oxime (99) into the nitrone (100) and subsequent ozonolysis. The nitro sugar (101) was obtained as a single anomer with α-configuration.

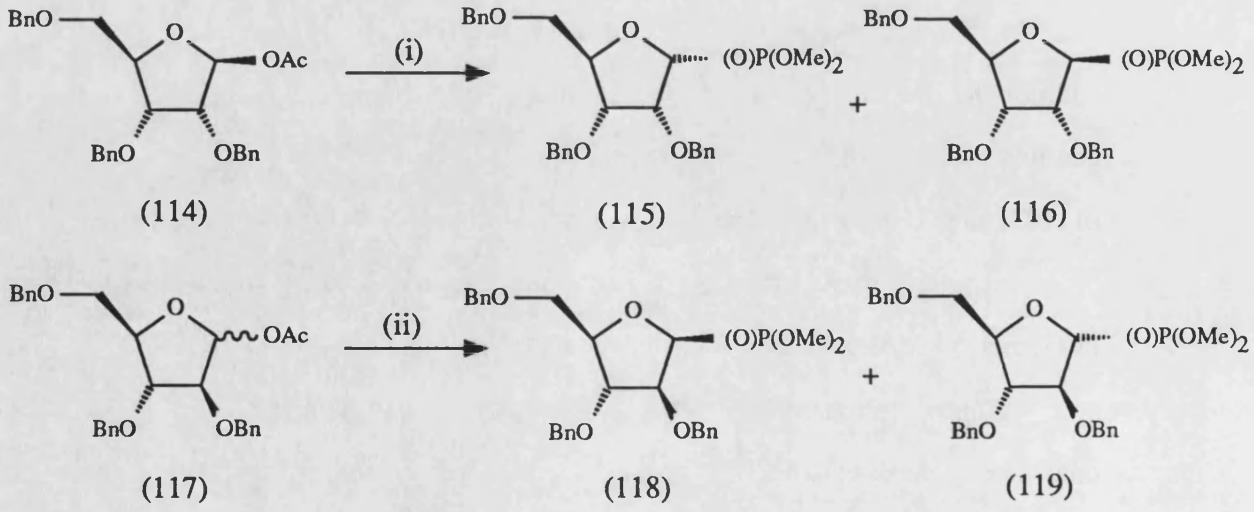
In the presence of 0.25 eq. of Bu_4NF in THF, (101) underwent a Michael addition to the dibenzyl vinyl phosphonate (102) to give after hydrolysis the addition product (103) as a mixture of anomers (α/β ratio 38:62). Hydrogenolysis of (103) gave (104) an isosteric analogue of D-fructose 1-phosphate (**Scheme 21**).

Similarly prepared were the isosteric analogues of D-ribulose 1-phosphate (105) and D-sedoheptulose 1,7-bisphosphate (106).

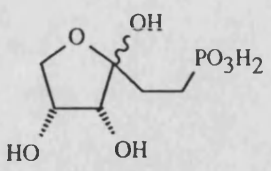
Another problem addressed by Vasella was the synthesis of compounds containing a phosphonate group at the anomeric centre. Initial attempts by Paulsen *et al.* had proved unsuccessful. The reaction of tetra-O-acetyl α-D-glucopyranosyl bromide (107) with triethyl phosphite did not yield the anomeric phosphonate but



Scheme 23. *Reagents and conditions:* (i) *t*-BuO^-K^+, $(EtO)_2P(O)H$, 18-crown-6, THF, hν, -40°- rt (72%); (ii) AIBN, Bu_3SnH, PhMe, reflux, 3h (78%); (iii) TMSBr, CH_2Cl_2, rt, 2h; (iv) H_2O, 50°, 4h (88%).



Scheme 24. *Reagents and conditions:* (i) $(MeO)_3P$, TMSOTf, CH_2Cl_2 (94%); (ii) $(MeO)_3P$, TMSOTf, CH_2Cl_2 (95%).



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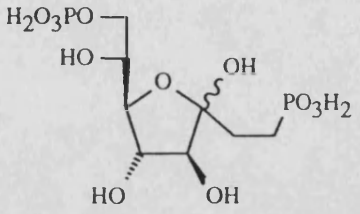


(106)

gave instead the hexenopyranose (108)[38] (**Scheme 22**).

Russell and Hershburger[39] had reported that the reaction of dialkyl phosphite anions with geminal chloro- or arylsulphonyl-nitroalkanes gave α-nitro-alkylphosphonates *via* a free radical chain mechanism. Meuwly and Vasella extended this reaction to the corresponding anomeric nitro derivatives of aldoses[40].

Thus treatment of nitrosulphone mannofuranose (109) with three equiv. of $KP(O)(OEt)_2$ in THF under irradiation gave the nitrile (110) (11%) and the nitro phosphonate (111) (61%). The formation of the phosphonate together with the nitrile was interpreted as the result of single electron transfer, leading to the phosphonate, competing with nucleophilic attack which leads to the nitrile.

The reductive denitration of the 1-C-nitroglycosyl phosphonate (111) with tributyltin hydride in the presence of AIBN gave the protected glycosylphosphonate (112) exclusively as the β-anomer, which was deprotected under standard conditions to afford the non-isosteric analogue (113) of β-D-mannofuranose-1-phosphate[40] (**Scheme 23**).

However, the scope of the multistep synthesis of the phosphonate (113) was limited and Meuwly and Vasella later reported[41] an improved route to glycosylphosphonates. The scheme was based on the known reactions of carbocations with trialkyl phosphites leading to dialkyl phosphonates[42]. It was postulated that 1-O-acyl-glycoses should react via the corresponding oxonium ions

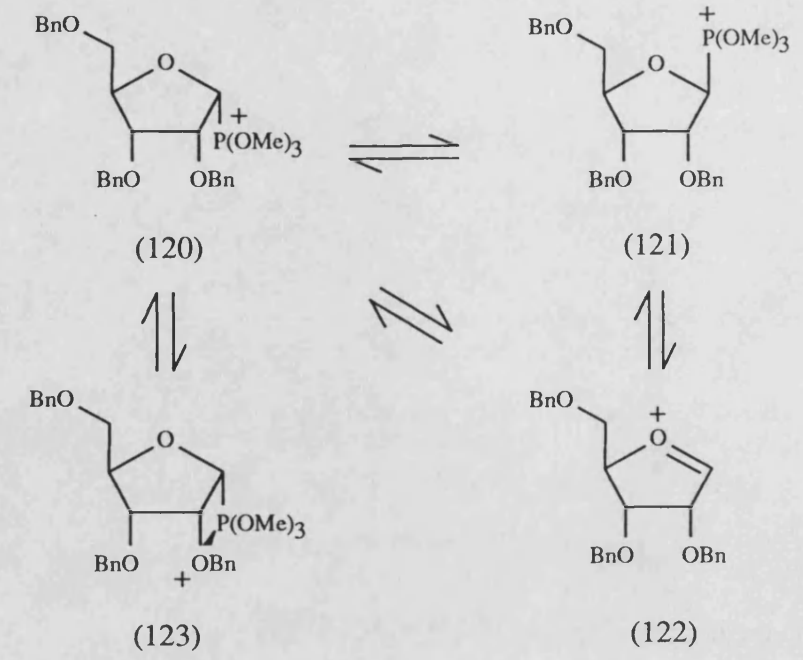
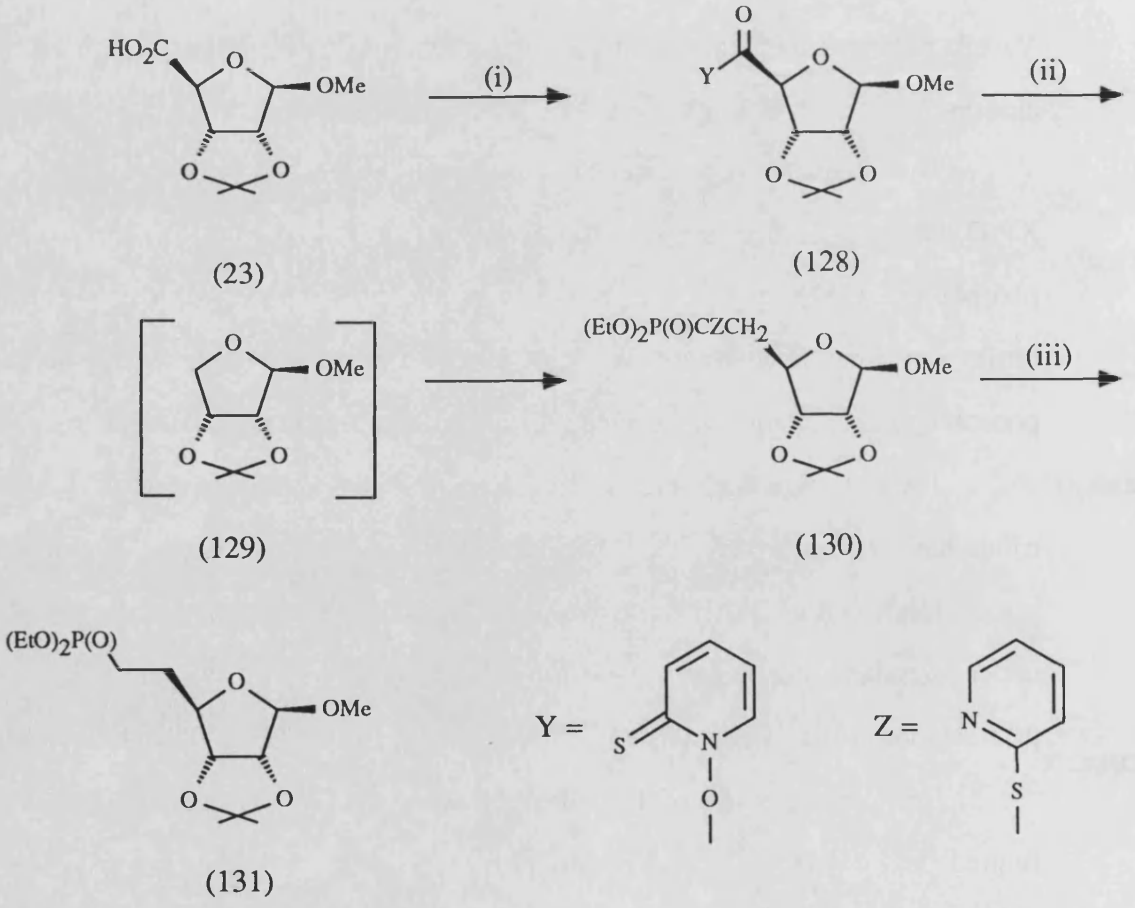


Figure 1.



Scheme 25. *Reagents and conditions:* (i) 2-mercaptopyridine N-methyl morpholine, THF, 0°, 1h; (ii) CH_2=CH(O)PO(OEt)$_2$, hv, THF, 0°, 30min; (iii) AIBN, Bu_3SnH, PhH.

to give glycosylphosphonates.

The reaction of acetyl β-D-ribofuranose (114) with trimethyl phosphite catalysed with trimethylsilyl trifluoromethanesulphonate gave the 1,2-cis configurated α-D-ribofuranosyl phosphonate (115) (88%) and the β-anomer (116) (6%).

Similarly, acetyl α/β-D-arabinofuranose (117) under the same conditions afforded predominantly the 1,2-cis configurated β-D-arabinofuranosyl phosphonate (118) (86%) and the α-anomer (119) (9%) (**Scheme 24**).

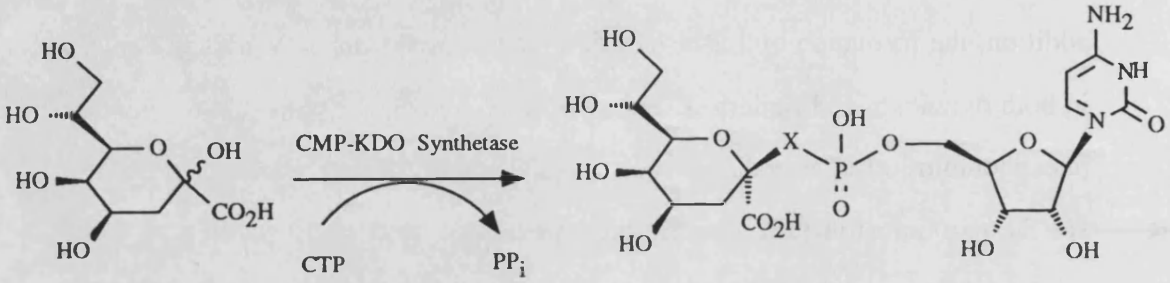
The stereochemistry of this Michaelis-Arbuzov type reaction does not depend on the anomeric configuration of the starting 1-O-acetyl glycose. In addition, the formation of 1,2-cis configurated phosphonates was almost exclusive in both pyranoses and furanoses. This was explained on the basis that the anomeric phosphonium-ion intermediates such as (120) and (121) are in direct or indirect, *via* the oxonium ion (122), equilibrium with each other and that the C-2 alkoxy group stabilises the 1,2-cis isomers by co-ordination with the phosphonium centre (123) (**Figure 1**).

The glycosyl phosphonates (115) and (118) were simply deprotected on treatment with bromotrimethylsilane followed by hydrogenolysis to give α-D-ribofuranosyl phosphonic acid (124) and β-D-arabinofuranosyl phosphonic acid (125) respectively.

Similarly prepared were α-D-glucopyranosyl phosphonate (126) and β-D-mannopyranosyl phosphonate (127).

Géro *et al.* utilised their previously developed methodology[43] for the formation of C(4) carbon radicals of pentoses for the synthesis of isosteric phosphonates[44].

The ribofuranuronic acid derivative (23)[16] was coupled with 2-mercaptopyridine N-oxide to give the 2-thiopyridone derivative (128). Irradiation with tungsten light efficiently produced the C(4) radical (129) which

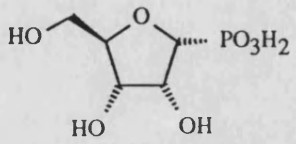


(132)

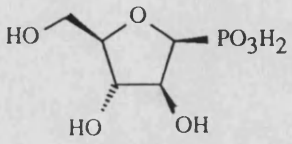
(133) X = CH_2

(134) X = O

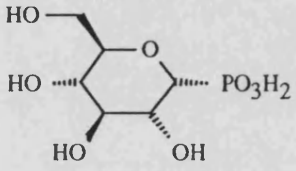
Figure 2.



(124)



(125)



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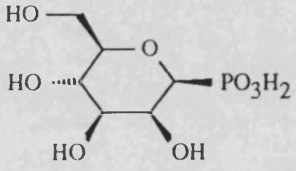
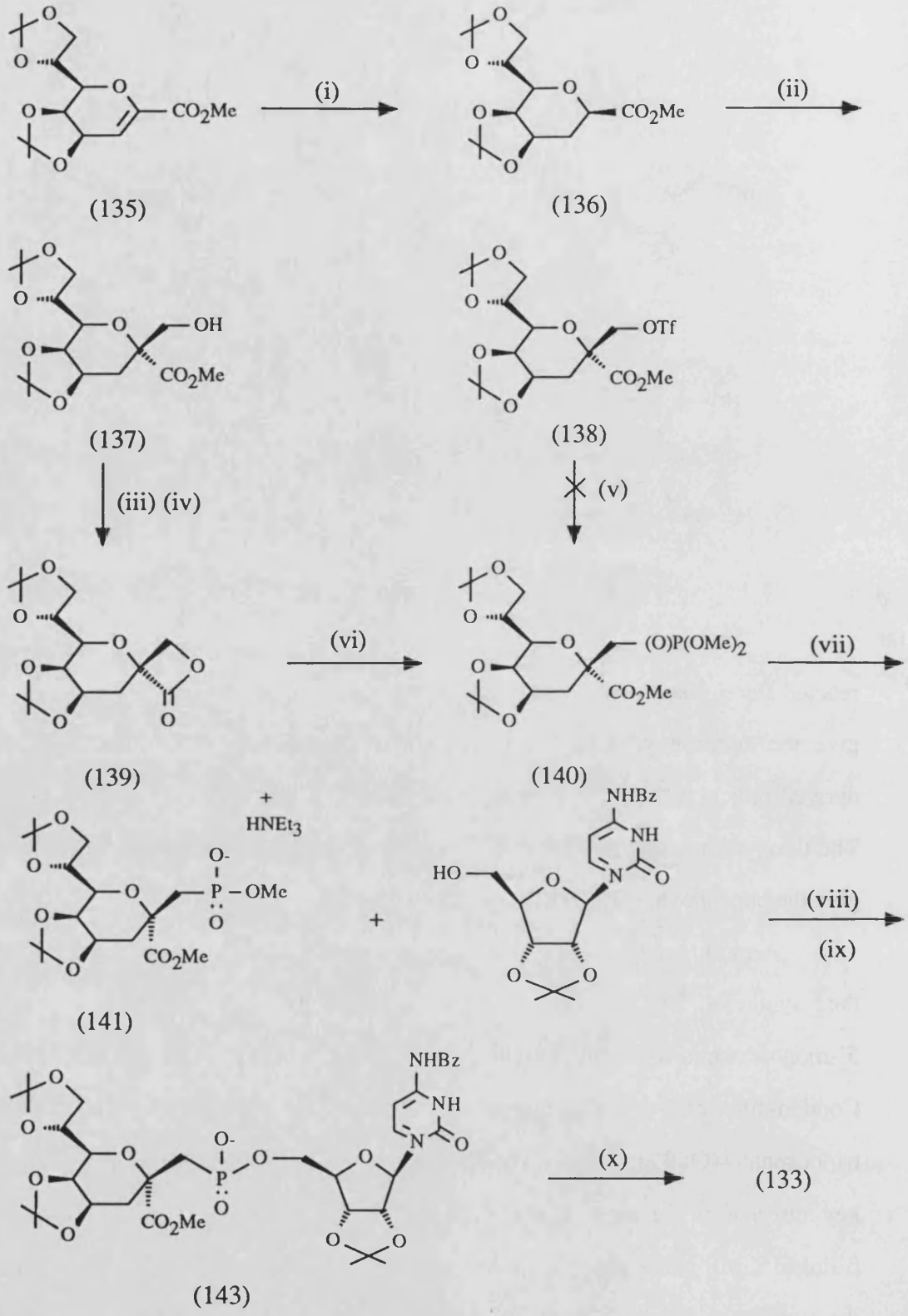


(127)

reacted stereospecifically with the activated alkene, diethyl vinyl phosphonate, to give the addition product (130) as a single stereoisomer. The chirality of the derived radical adduct had been controlled by the steric bulk of the acetal group. The thiopyridone function could be removed efficiently with tributyltin hydride to give the phosphonate (131) (**Scheme 25**).

A modified Michaelis-Arbuzov reaction was used by Norbeck *et al.*[45] for the synthesis of an isosteric phosphonate analogue (133) of cytidine 5'-monophospho-3-deoxy D-manno-2-octulosonic acid (CMP-KDO) (134). Condensation of 3-deoxy-D-manno-2-octulosonic acid (KDO) (132) with cytidine triphosphate (CTP) to form CMP-KDO is catalysed by CMP-KDO synthetase, a key enzyme in bacterial lipopolysaccharide biosynthesis (**Figure 2**). A stable β-linked CMP-KDO phosphonate analogue (133) could conceivably inhibit both the synthetase and the subsequent transferase that catalyse displacement of CMP by a lipid A precursor.

The hydrogenation of a glycal (135) over Raney nickel proceeded exclusively from the unencumbered α-face to yield (136). In contrast, the addition of gaseous formaldehyde occurred predominantly (5.1:1) from the β-face to afford largely the required C-glycoside (137) and its chromatographically separable



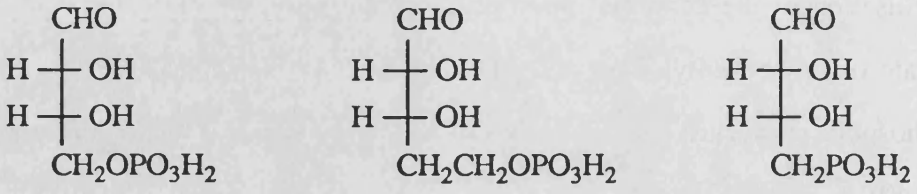
Scheme 26. *Reagents and conditions:* (i) H_2, Raney-Ni, rt, 30min (88%); (ii) CH_2O, LDA, THF, -78°, 45min (91%); (iii) aq. LiOH, MeOH, 3h, rt, H_3O^+; (iv) Et_3N, $C_6H_5SO_2Cl$, CH_2Cl_2, rt, 1h, (78%); (v) $(MeO)_3P$, reflux (vi) $(MeO)_3P$, 100°, 24h (37%); (vii) Et_3N, C_6H_5SH, THF, rt, 20h, (90%); (viii) Ph_3P, DEAD, THF, rt, 24h; (ix) Et_3N, C_6H_5SH, THF, rt, 24h (75%); (x) aq. NaOH, MeOH, rt, 3h, H^+ resin (93%).

isomer. Displacement of the alcohol moiety in (137) by a phosphorus nucleophile proved difficult. Despite the unrivalled ability of triflate esters to act as leaving groups, the triflate (138) was inert to refluxing trimethyl phosphite. However, utilisation of the carboxyl group as a leaving group divested the SN2 transition state of its neopentyl character. Thus, heating the β-lactone (139) with trimethyl phosphite in a glass pressure tube afforded a moderate yield of the phosphonate (140).

Selective demethylation of the phosphate ester with thiophenoxide gave (141) which was coupled with the known cytidine derivative (142)[46] using modified Mitsunobu conditions. Removal of the protecting groups from (143) under standard conditions smoothly yielded the the isosteric phosphonate analogue (133) (**Scheme 26**).

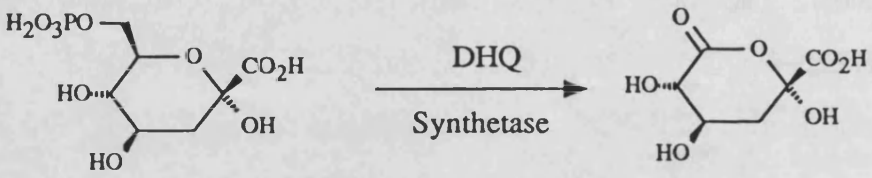
In a purified CMP-KDO synthetase assay([KDO]=1mM, [CTP]=0.5mM), the phosphonate was a modest inhibitor, with I_{50}=4.1mM. In a permeabilised whole cell assay, the phosphonate (133) did not significantly inhibit transfer of KDO from CMP to lipid A precursor.

Although it is not catalytically advantageous for the enzyme to bind it's substrates or products strongly, the K_m's for KDO and CTP, 31 and 11μM respectively, suggests that the synthetase binds the CMP-KDO phosphonate (133) relatively weakly.



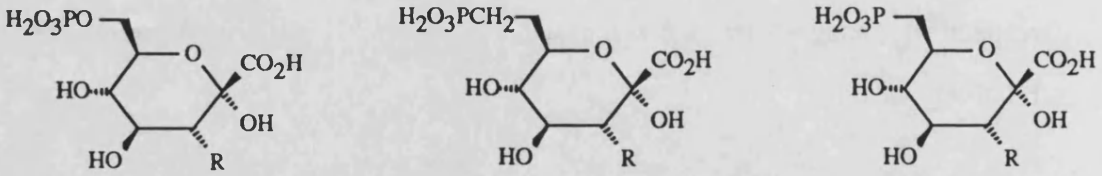
(144) (145) (146)

Figure 3.



(147) (148)

Figure 4.



(147) R = H
(149) R = OH

(150) R = H
(151) R = OH

(152) R = H
(153) R = OH

Figure 5.

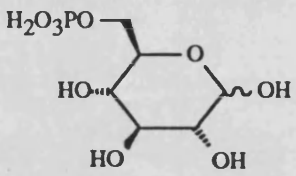
ISOSTERIC VS NON-ISOSTERIC PHOSPHONATES

In general, isosteric phosphonate analogues of carbohydrate phosphates are metabolised in all respects like the natural substrates, except for reactions in which the phosphate acts as a leaving group. However, in many instances the enzymes bind the isosteric phosphonate analogues several times more weakly than the naturally occurring phosphates. This has been attributed to small differences in bonding geometries, steric interactions with the methylene hydrogens, deletion of a possible hydrogen bond to the phosphate oxygen linkage and incomplete ionisation of the phosphonate diacids. Indeed, recent reports underscore the unpredictable behaviour of phosphonate analogues with enzymes. Few direct comparisons of isosteric or homophosphonates have been made with the corresponding non-isosteric phosphonates.

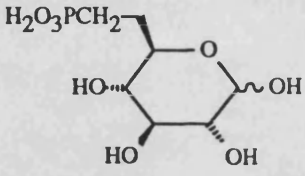
Level *et al.*[47] compared the isosteric analogue (145) of D-erythrose 4-phosphate (144) with the non-isosteric analogue (146) (**Figure 3**).

While the V_{max} of the isosteric and non-isosteric analogues were 29% and 5%, respectively, of the V_{max} of the natural substrate with *Escherichia Coli*, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase, the V_{max} was the same for all of the compounds with yeast transaldolase.

The same authors investigated the 3-dehydroquinate synthetase catalysed transformation of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP) (147) to dehydroquinate (DHQ) (148) and inorganic phosphate)[48] (**Figure 4**). Both the isosteric and non-isosteric analogues of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (147) and D-gluco-heptulosonic acid 7-phosphate (149) were synthesised (**Figure 5**). The isosteric analogues (150) and (151) behaved as weak competitive inhibitors with respective K_i values[49] of 0.26 and 1.2mM. However, both the non-isosteric analogues (152) and (153) were strong competitive inhibitors with K_i values of 2.5 and 5μM respectively. Remarkably, the



(154)



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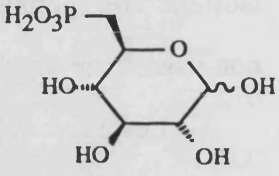


(156)

Figure 6.

non-isosteric 3-deoxy-D-arabino heptulosonic acid 7-phosphonate (152) bound 20 times more strongly to dehydroquinate synthetase than the natural substrate whose K_M value was 50μM..

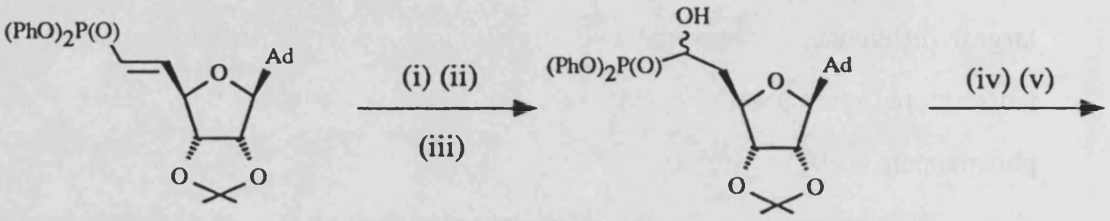
The phosphonate (152) and isosteric phosphonate (150) analogues were independently synthesised, and their enzymatic activity re-evaluated using rigorously purified *E. coli* enzyme by Frost *et al.*[50].

The Michaelis constant (K_M=18μM) and the inhibition constant (K_i=1.1μM) for inhibition by phosphonate (152) were roughly halved. However the largest difference between the two studies was the measured inhibition by the isosteric phosphonate (150). In the later work no inhibition by the isosteric phosphonate (150) was observed.

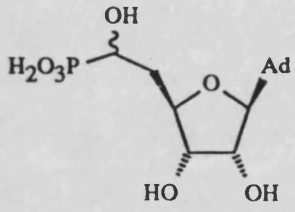
The absence of inhibition of substrate binding by an isosteric phosphonate analogue is precedented[51]. One explanation may be incomplete dissociation of the phosphonic acid. Phosphonates which are not appropriately substituted by acceptor groups are less acidic than phosphates[52]. Other explanations centre on the C-O-P oxygen of the phosphate ester. Substitution of a methylene for this oxygen may lead to adverse steric interactions with the enzyme active site, although the position of the methylene group of the isosteric analogue (150) is occupied by the entire phosphonate moiety of phosphonate (152). Alternatively, the C-O-P oxygen of DAHP may play an important role as a Lewis base in binding to the active site of DHQ synthetase. Such interaction would not be possible with the methylene group of isosteric (150) but might be possible with the phosphonate moiety of (152).

Leval *et al.*[48] also synthesised the isosteric (155) and non-isosteric (156) phosphonate analogues of D-glucose 6-phosphate (154) (**Figure 6**).

Phosphonate analogue (156), in contrast to the isosteric phosphonate derivative, behaved as a competitive inhibitor of D-glucose 6-phosphate dehydrogenase with a K_i of 0.4mM.



(157) (158)



(159)

Scheme 27. *Reagents and conditions:* (i) BH_3.THF, rt, 18h; (ii) 30% H_2O_2, 2M NaOH, 0° - rt, 5h; (iii) Dowex 50 (H^+), aq. MeOH (71%); (iv) AcOH, aq. MeOH, 90°, 1h (v) phosphodiesterase (74%)

The polar properties of non-isosteric glycosyl phosphonates[40] were shown to be similar to those of the corresponding glycosyl phosphates by Briner and Vasella[53]. Their polar character was measured on the basis of their pKa values.

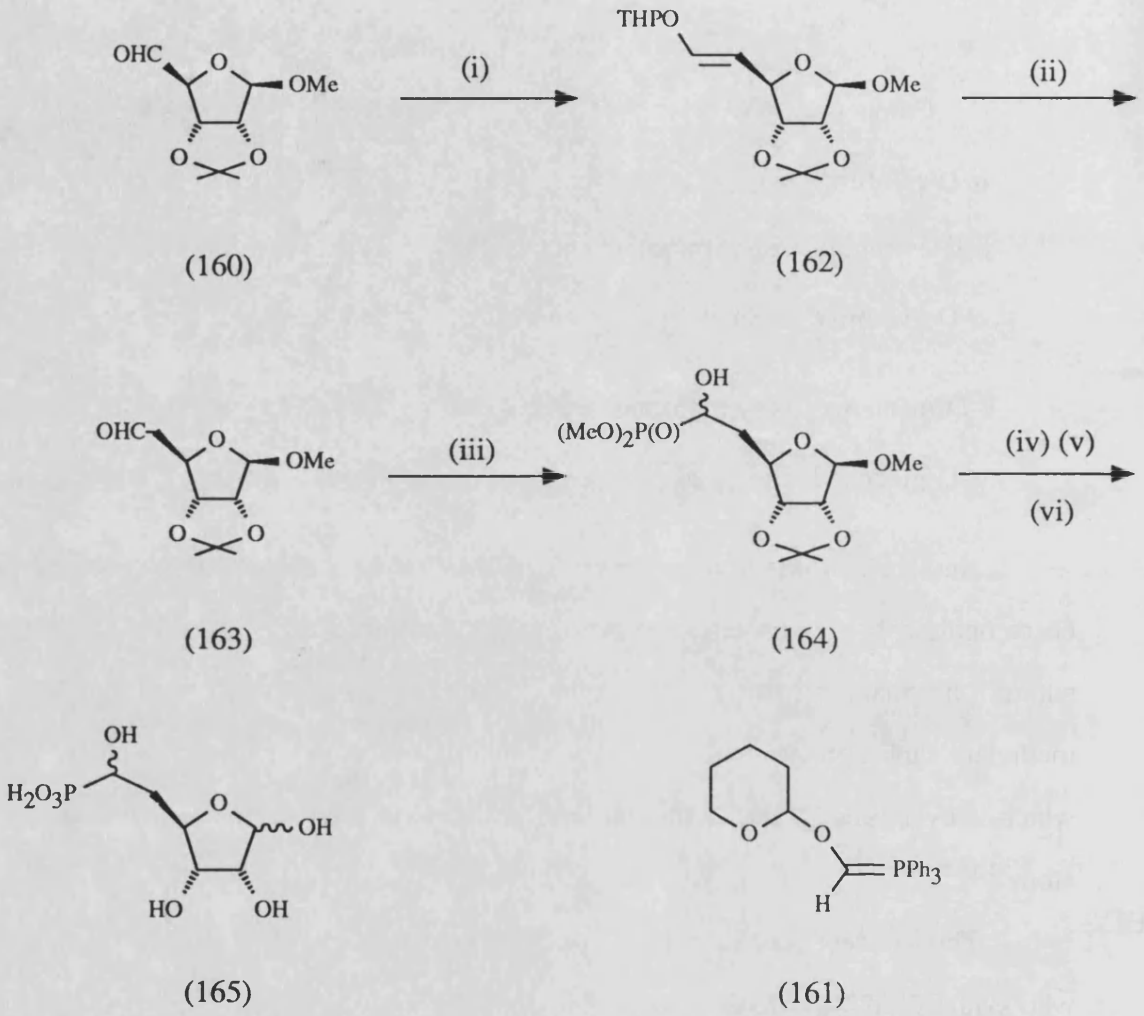
Compound	pKa(1)	pKa(2)
α-D-ribofuranosyl phosphonic acid (115)	2.72	6.50
β-D-arabinofuranosyl phosphonic acid (118)	2.63	6.10
α-D-glucopyranosyl phosphonic acid (126)	2.77	6.38
β-D-mannopyranosyl phosphonic acid (127)	2.60	6.12
α-D-glucose 1-phosphate	—	6.22

Another possible way to overcome the failure of isosteric phosphonates to be recognised by certain enzymes is to incorporate the binding capability of the natural phosphate into the methylenephosphonate. One example is the failure of methylene diphosphonic acid to serve as an inhibitor of a variety pyrophosphatases which is overcome by the incorporation of a hydroxyl group α to the phosphorus atom[54,55].

This concept was extended to the carbohydrate series by Hampton *et al.*[56] who synthesised the α-hydroxy analogue of AMP. Their approach began from the α/β-unsaturated phosphonate (157) synthesised from the corresponding aldehyde using the methodology developed by Jones and Moffatt[11].

Hydroboration of (157), with a borane-THF complex, followed by oxidation with hydrogen peroxide and sodium hydroxide led to the formation of the α-hydroxy phosphonate (158) as a mixture of diastereomers. The remaining phenyl ester was cleaved on treatment with snake venom phosphodiesterase, acid hydrolysis then afforded the α-hydroxy phosphonate AMP analogue (159) **(Scheme 27)**.

Both of the 6'-epimers of (159) were substrates of AMP aminohydrolase



Scheme 28. *Reagents and conditions:* (i) $THPOCH_2{}^+PPh_3Cl^-$, ^nBuLi, THF, rt, 20h, (65%); (ii) 80% AcOH, 50°, 5h, (70%); (iii) $(MeO)_2P(O)H$, NaOMe, PhH, rt, 16h, (71%); (iv) TMSBr, Et_3N, CCl_4, rt, 12h; (v) H_2O, 1,4-dioxane, rt, 12h, (77%); (vi) Dowex 50 (H^+), H_2O

from rabbit muscle[56]. The V_{max} of the more active epimer was essentially the same as that of AMP itself and five times greater than the simple isosteric phosphonate analogue[57].

Presumably the enhanced substrate capability results from an ability of the 6'-oxygen of the more active epimer of (159) to perform some function of the oxygen atom linkage of AMP. The fact that the V_{max} value of the less active epimer is eightfold less than that of the isosteric phosphonate analogue could indicate an insufficiency of space in the enzyme substrate complex to accommodate the hydroxy group of that epimer.

An identical approach to that of Hampton was employed by Engel *et al.* to prepare α-hydroxy phosphonate analogues of a series of nucleotide and carbohydrate related phosphate esters[58].

An alternative approach to α-hydroxy phosphonates, other than *via* a substituted vinyl phosphonate and subsequent hydration, was undertaken by Engel *et al.*[59] This was necessitated by the inability to synthesise a substituted vinyl phosphonate diester from the D-ribose derived aldehyde (160). As previously reported[60], reaction of an aldehyde bearing an acetal function with a phosphonate anion reagent resulted in a multitude of side reactions involving the acetal linkage. The successful synthesis incorporated, at an early stage, the proper number of carbon atoms and functionality with an aldehyde function at the position ultimately to bear the phosphorus and hydroxy groups.

Thus, reaction of the aldehyde (160) with the ylid (161)[61] afforded the enol ether (162) as approximately an equal distribution of E and Z isomers. Selective cleavage of the enol ether was achieved on warming with 80% aq. acetic acid. The resulting aldehyde (163) underwent an Abramov reaction with dimethyl phosphite in benzene with addition of sodium methoxide for anion generation. The α-hydroxy dimethyl phosphonate (164) produced was then deprotected in the usual manner to give the α-hydroxy phosphonic acid analogue (165) of D-ribose

5-phosphate (**Scheme 28**).

The reaction of dimethyl phosphite with the prochiral aldehyde carbon of (163) generates a new chiral centre, and the formation of a pair of diastereomers would be expected. However, no positive evidence for the presence of two diastereomers was found in the ^{1}H n.m.r. spectrum, nor could any separation of materials be observed under any chromatographic conditions used.

METABOLISM OF GLUCOSE

Glycolysis

The generation of metabolic energy via glycolysis is a nearly universal pathway in biological systems. Glycolysis is the sequence of reactions that converts glucose into pyruvate with the concomitant production of ATP.

The first stage involves the conversion of glucose into fructose 1,6-bisphosphate in three steps: a phosphorylation, an isomerization and a second phosphorylation reaction. The strategy of these initial steps in glycolysis is to form a compound that can be readily cleaved into phosphorylated three carbon units. Energy is subsequently abstracted from these three carbon units.

The net reaction in the transformation of glucose into pyruvate is

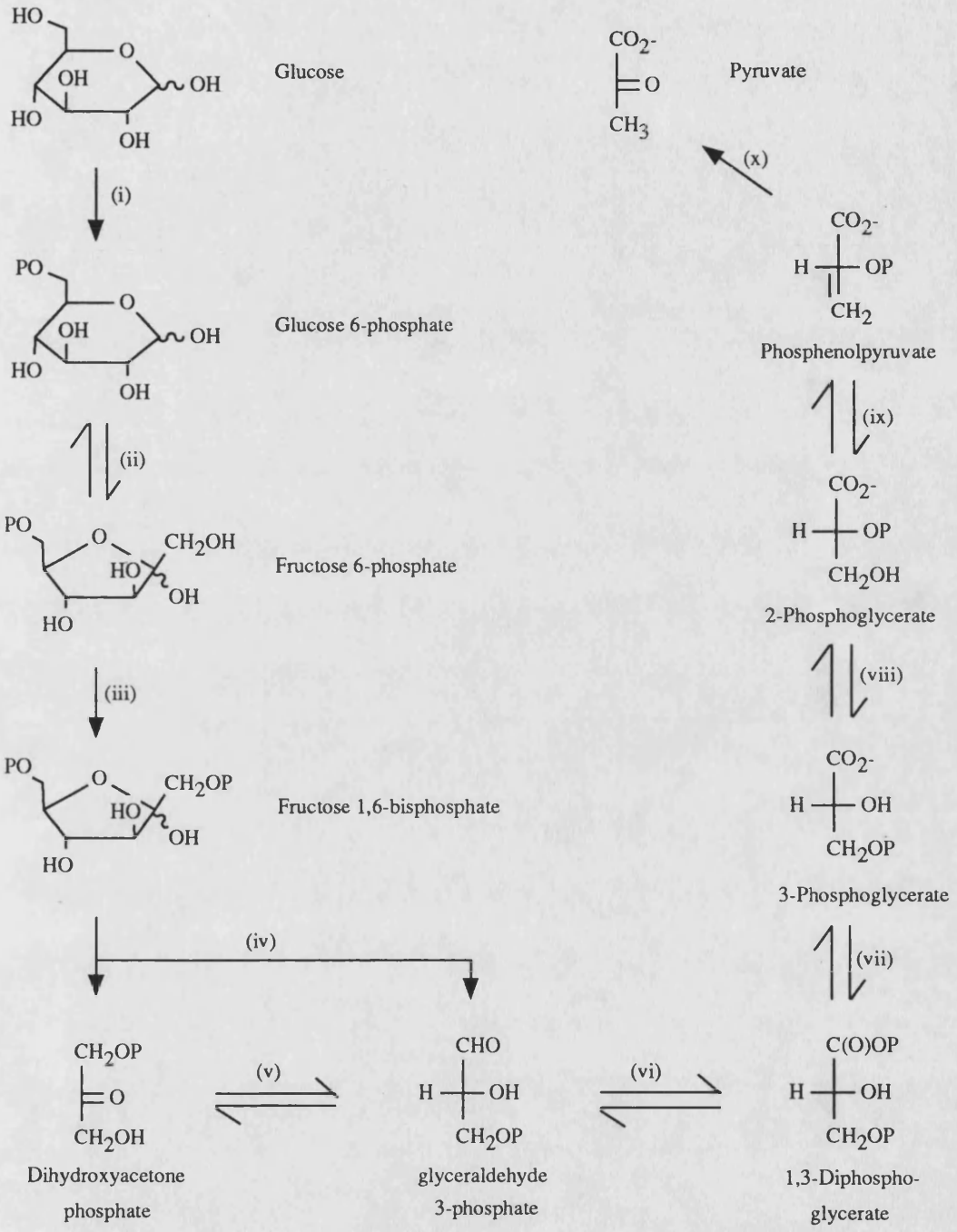
$$\text{Glucose} + 2P_i + 2ADP + 2NAD^+ \longrightarrow 2\text{Pyruvate} + 2ATP + 2NADH + 2H^+ + 2H_2O$$

Thus, two molecules of ATP are generated in the conversion of glucose into pyruvate. However, only a small fraction of the energy of glucose is released in its anaerobic conversion into pyruvate. Much more energy can be extracted aerobically by means of the citric acid cycle and the electron transport chain. The entry point to this oxidative pathway is acetyl co-enzyme A, which is formed inside mitochondria by the oxidative decarboxylation of pyruvate.

$$\text{Pyruvate} + NAD^+ + \text{Co A} \longrightarrow \text{acetyl Co A} + CO_2 + NADH$$

The glycolytic pathway has a dual role: it degrades glucose to generate ATP and it provides building blocks for biosynthetic reactions, such as the

Glycolytic Pathway



Enzymes: (i) Hexokinase (ATP → ADP); (ii) Phosphoglucoisomerase; (iii) Phosphofructokinase (ATP → ADP); (iv) Aldolase; (v) Triose phosphate isomerase (vi) Glyceraldehyde 3-phosphate dehydrogenase (NAD^+ → NADH); (vii) Phosphoglycerate kinase (ADP → ATP); (viii) Phosphoglycerate mutase; (ix) Enolase (x) Pyruvate kinase (ADP → ATP).

formation of long-chain fatty acids. The rate of conversion of glucose into pyruvate

is regulated to meet these two major cellular needs.

In metabolic pathways, enzymes catalysing essentially irreversible reactions are potential sites of control. In glycolysis three reactions, catalysed by hexokinase, phosphofructokinase (PFK), and pyruvate kinase are virtually irreversible. Of these PFK is the most important control element, since the first irreversible reaction unique to the glycolytic pathway is the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate.

PFK is inhibited by high levels of ATP, which lowers the affinity of the enzyme for fructose 6-phosphate. This allosteric effect is evoked by the binding of ATP to a highly specific regulatory site that is distinct from the catalytic site. This inhibitory action of ATP is reversed by AMP, so the activity of the enzyme increases when the ATP/AMP ratio is lowered. In other words, glycolysis is stimulated when the energy charge of the cell is low. As glycolysis provides pyruvate for the synthesis of acetyl Co-A used in biosynthesis, PFK is also regulated by a signal indicating whether building blocks are abundant or scarce. Citrate, an early intermediate in the citric acid cycle, inhibits PFK by enhancing the inhibitory effect of ATP. A high level of citrate indicates that biosynthetic precursors are abundant and thus, additional glucose should not be degraded for this purpose.

Glycogen Metabolism

Glucose is readily stored as glycogen, a very large branched polymer of glucose residues. The presence of glycogen greatly increases the amount of glucose that is immediately available between meals and during muscular activity.

The synthesis and degradation of glycogen are important because they regulate the blood glucose level and provide a reservoir of glucose for strenuous muscular activity. Glycogen is synthesised and degraded by two distinct pathways which affords greater flexibility, both in energetics and control.

Glycogen is broken-down by glycogen phosphorylase to glucose 6-phosphate and glycogen with n-1 residues. Contrarily, glycogen is synthesised by glycogen synthetase which adds a glycosyl residue, from the glucose donor uridine diphosphate glucose, to the glycogen polymer. Glycogen synthesis and degradation are co-ordinately controlled so that glycogen synthetase is nearly inactive when phosphorylase is fully active and vice versa.

Glycogen metabolism is profoundly affected by specific hormones. The polypeptide hormone insulin increases the capacity of the liver to synthesise glycogen, and high levels of insulin in the blood signify the fed state. Conversely, glucagon has the opposite effect to that of insulin. This hormone increases blood sugar levels by stimulating the breakdown of glycogen in the liver. Glucagon does not enter the target cell but rather stimulates adenylase cyclase, which catalyses the formation of cyclic AMP from ATP. Cyclic AMP then activates a protein kinase which phosphorylates both glycogen synthetase (rendering it inactive) and phosphorylase kinase (rendering it active). In this way, cyclic AMP stimulates glycogen breakdown and stops glycogen synthesis. Cyclic AMP acts as a signal in the sense defined by Stryer[62]. Although formed from ATP, a ubiquitous molecule at the centre of metabolic transformations, it is not itself part of a major metabolic pathway. It is used only as an integrator of metabolism, not as a biosynthetic

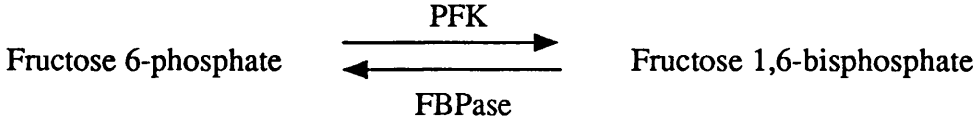
precursor or intermediate in energy production. Hence, its concentration can be independently controlled. Cyclic AMP is a hunger signal which signifies the absence of glucose.

Gluconeogenesis

The process of glucose synthesis from non-carbohydrate precursors is called gluconeogenesis. This metabolic pathway is very important because certain tissues, such as the brain are highly dependant on glucose as the primary fuel. Gluconeogenesis also plays an essential role during periods of intense exercise. The major non-carbohydrate precursors of glucose are lactate, amino acids and glycerol. Lactate is formed by active skeletal muscle when the rate of glycolysis exceeds the metabolic rate of the citric acid cycle and the respiratory chain. Gluconeogenesis occurs in the liver and helps to maintain the glucose level in the blood so that the brain and muscle can extract sufficient glucose from it to meet their metabolic demands.

Gluconeogenesis is not an exact reversal of glycolysis as the thermodynamic equilibrium of the reactions catalysed by hexokinase, PFK and pyruvate kinase lies far on the side of pyruvate formation. Hence, in gluconeogenesis, these virtually irreversible reactions of glycolysis are bypassed.

Fructose 1,6-bisphosphate is hydrolysed to fructose 6-phosphate by fructose 1,6-bisphosphatase (FBPase) in an exothermic process.

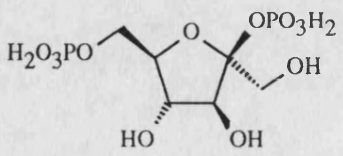


Regulation of Glycolysis and Gluconeogenesis

Glycolysis and gluconeogenesis are co-ordinated so that one pathway is relatively inactive while the other is highly active. The distinctive enzymes of each pathway are controlled so that both pathways cannot be highly active at the same time. For example, AMP stimulates PFK, whereas it inhibits FBPase. Citrate has a reverse effect on these enzymes.

Consequently, phosphorylation of fructose 6-phosphate, the rate limiting step of glycolysis, is enhanced when the energy charge of the cell is low. Conversely, fructose 1,6-bisphosphate is hydrolysed and gluconeogenesis is stimulated when the energy charge is high and citric acid cycle intermediates are abundant.

In 1980, Van Schaftingen, Hue and Hers discovered another low molecular weight stimulator of PFK from purified rat liver[63]. This stimulator showed extreme acid lability. In the presence of 0.01M HCl, half of the stimulator was destroyed within 15 minutes at 0°C. The reaction was characterized by the formation of fructose 6-phosphate together with an equivalent amount of reducing power and inorganic phosphate. Van Schaftingen *et al.* postulated correctly[64] that the stimulator of PFK was fructose 2,6-bisphosphate. NMR analysis later revealed that fructose 2,6-bisphosphate had the β-configuration (166).



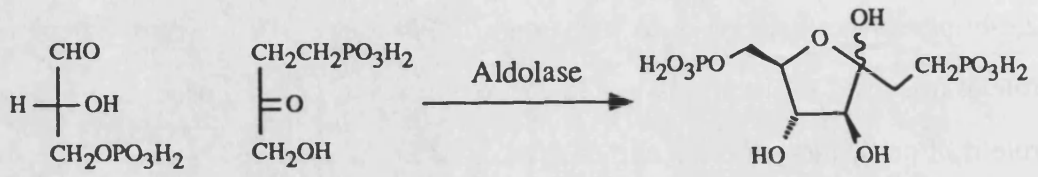
(166)

Fructose 2,6-bisphosphate now appears to be the most potent known positive effector of liver PFK[65], acting at concentrations 1000-fold smaller than

fructose 1,6-bisphosphate.

In addition to its stimulation of PFK, fructose 2,6-bisphosphate is a potent inhibitor of liver FBPase at micro-molar concentrations. The concentration of fructose 2,6-bisphosphate in the liver is dependent on nutritional state. It reaches 15-20μM after a glucose load and is decreased to very low levels during fasting, after glucagon treatment and in diabetes.

Fructose 2,6-bisphosphate is synthesised from fructose 6-phosphate and ATP by the enzyme 2-phosphofructokinase (PFK2), and it is hydrolysed into fructose 6-phosphate and Pi by fructose 2,6-bisphosphotase (FBPase2). PFK is inactivated and FBPase is activated by cyclic AMP dependant protein kinases and this effect explains the fact that glucagon causes the disappearance of fructose 2,6-bisphosphate. Because of its action on both PFK and FBPase, the most obvious role of fructose 2,6-bisphosphate is to control glycolysis and gluconeogenesis. This role is of particular importance in the liver in which both pathways can operate. In this respect, fructose 2,6-bisphosphate plays a similar but opposite role to cyclic AMP. Cyclic AMP has been called a hunger signal, which signifies the absence of glucose. In contrast, fructose 2,6-bisphosphate is the signal which signifies that glucose is abundant and can be freely used and that gluconeogenesis can be stopped.



(168) (169) (170)

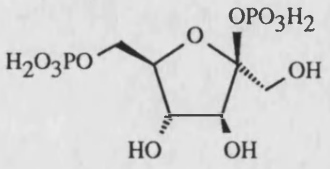
Figure 7.

SYNTHESIS OF FRUCTOSE PHOSPHATE ANALOGUES

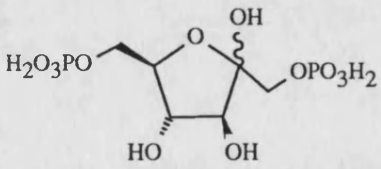
As previously described phosphate esters of D-fructose play an essential role in the regulation of glycolysis and gluconeogenesis. Obviously, analogues of these compounds possess intriguing possibilities for modes of action in both metabolic processes.

Analogues of fructose 1,6-bisphosphate (167) could act as competitive inhibitors of FBPase by occupation of the active site. For example, replacing the 1-phosphate group with a phosphonic acid should yield a compound, still recognizable by FBPase, that cannot be hydrolysed to fructose 6-phosphate.

Alternatively, analogues of fructose 2,6-bisphosphate (166), stable to both chemical and enzymic hydrolysis, could be synthesised which may still activate PFK and inhibit FBPase.



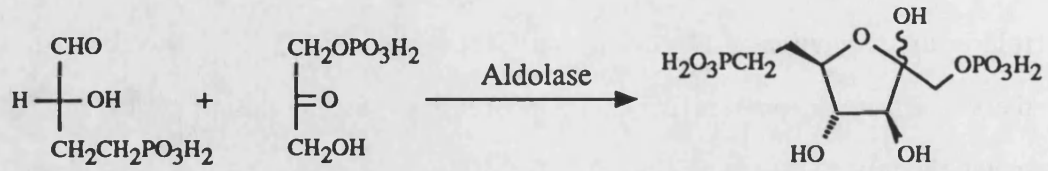
(166)



(167)

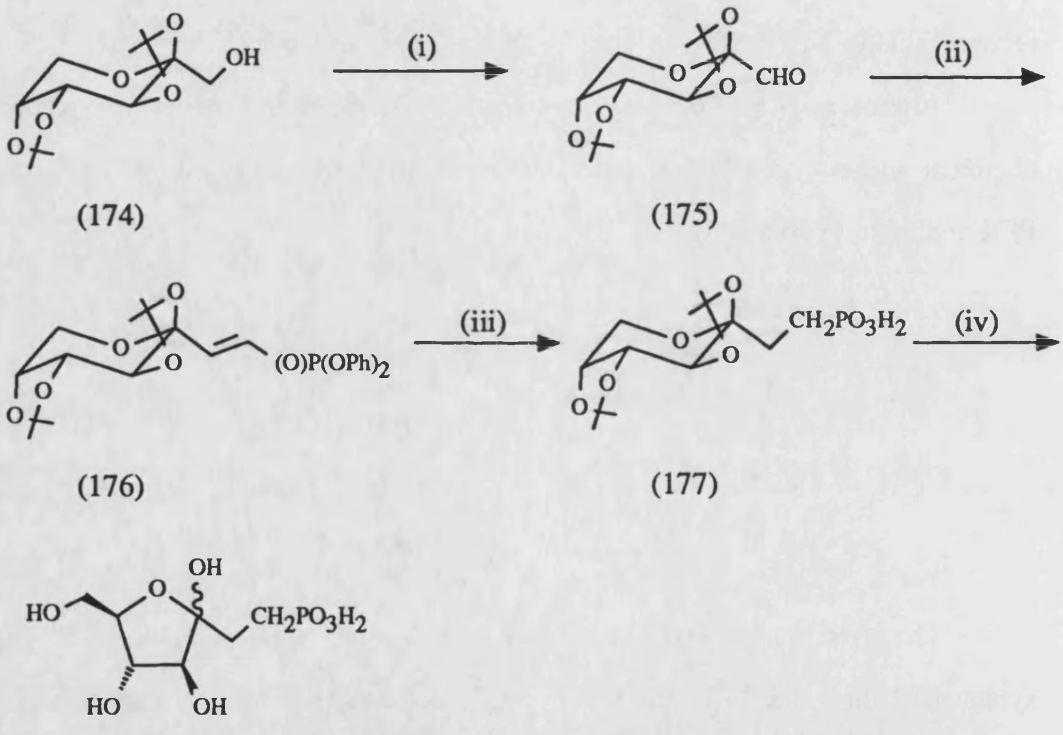
The first phosphonate analogue (170) of a fructose phosphate was synthesised enzymically by Stribling[66]. In gluconeogenesis the aldose catalysed condensation of glyceraldehyde 3-phosphate with dihydroxyacetone phosphate affords fructose 1,6-bisphosphate. The 1-phosphonomethyl analogue (170) of fructose 1,6-bisphosphate was synthesised by the aldol condensation of the isosteric phosphonate analogue (169) of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (168) catalysed by rabbit muscle aldolase (**Figure 7**).

The substitution of the isosteric phosphonic acid for the 1-phosphate group in fructose 1,6-bisphosphate abolished all measurable activity with FBPase, which hydrolyses the 1-phosphate of the natural substrate. The affinity of FBPase for the



(171) (172) (173)

Figure 8.



(104)

Scheme 29.*Reagents and conditions:* (i) DMSO, DCC, Py.HCl, rt, 24h (84%); (ii) $Ph_3P{=}CH(O)P(OPh)_2$, DMSO, rt, 48h (48%); (iii) H_2, PtO_2, EtOH, rt, 48h (84%); (iv) Dowex 50 (H^+), H_2O, 100°, 1h (89%).

phosphonate analogue was though decreased by approximately an order of magnitude with a K_i value of 70μM compared with K_M= 4μM for the natural substrate.

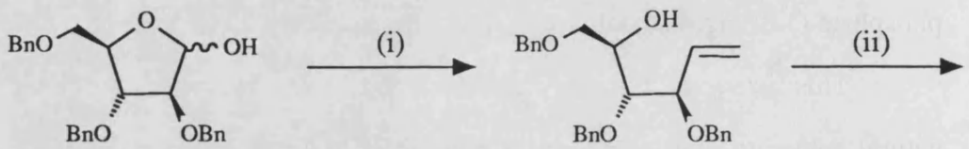
The same enzymic reaction was utilised by Jondorf *et al.*[67] to synthesise the analogue of fructose 1,6-bisphosphate in which the 6-phosphate group is replaced by an isosteric phosphonic acid. In this case, the isosteric phosphonate analogue (171) of glyceraldehyde 3-phosphate was condensed with dihydroxyacetone phosphate (172) again catalysed by rabbit muscle aldolase (**Figure 8**).

This isosteric phosphonate analogue (173) inhibited hydrolysis of the natural substrate with an apparent K_i of 150μM, nearly two orders of magnitude greater than K_M of the substrate.

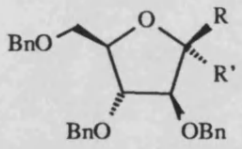
An analogue with an isosteric phosphonic acid replacing the 1-phosphate group was first synthesised by standard chemical techniques by Engel *et al.*[68]. Incorporation of the methylene phosphonate moiety was achieved by the Wittig methodology of Jones[11]. Thus, Swern oxidation of readily available 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (174) gave the primary aldehyde (175). The reaction of the primary aldehyde with diphenyl triphenylphosphoranylidenemethylphosphonate afforded exclusively the trans vinyl phosphonate (176). Hydrogenation over platinum oxide reduced the double bond and cleaved the phenyl esters. The phosphonic acid (177) was then simply deprotected on treatment with an acidic ion exchange resin to afford the isosteric analogue (104) of fructose 1-phosphate (**Scheme 29**).

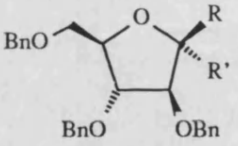
As previously described the isosteric analogue (104) has also been prepared by Vasella[36] utilising the Michael addition to a vinyl phosphonate of the nitro sugar (101) followed by hydrolysis of the anomeric nitro group (**Scheme 21**).

Maryanoff *et al.* synthesised α- (178) and β-D-arabinose 1,5-bisphosphate (179) as analogues of fructose 2,6-bisphosphate lacking the anomeric hydroxymethyl substituent.



(180) (181)





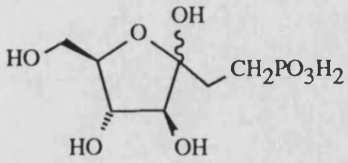
(182) R = H, R' = CH_2Br

(183) R = CH_2Br, R' = H

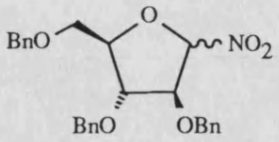
(184) R = H, R' = CH_2HgBr

(185) R CH_2HgBr, R' = H

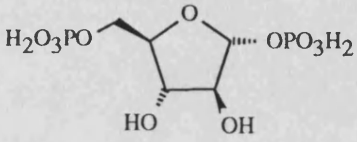
Scheme 30. *Reagents and conditions:* (i) $Ph_3P{=}CH_2$, THF, rt, 15min (68%); (ii) NBS, CH_2Cl_2, 0°, 15min



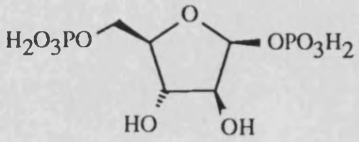
(104)



(101)



(178)



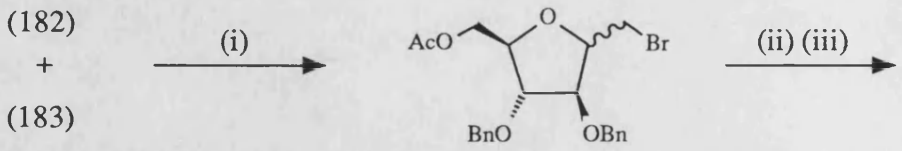
(179)

As competitive inhibitors of FBPase the α- and β-anomers had K_i values of 35 and 3.4μM respectively compared to K_i= 0.15μM for fructose 2,6-bisphosphate. As allosteric activators of PFK fructose 2,6-bisphosphate, the α-, and the β-anomer had half maximal concentrations of 0.05, 0.5 and 1.0μM respectively.

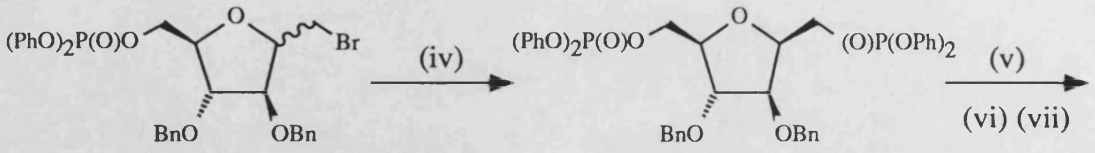
These results suggest that the anomeric hydroxymethyl group is not essential for binding to the enzyme to take place. Interestingly, neither (178) or (179) were substrates for FBPase2 the enzyme that degrades fructose 2,6-bisphosphate. This suggests that the arabinose analogues may have increased biological lifetime and thus, enhanced activity although, they still possess the very acid labile anomeric phosphate moiety.

Since arabinose 1,5-bisphosphate was shown to be an inhibitor of FBPase, and bearing in mind its acid lability, two groups undertook the synthesis of stable isosteric phosphonate analogues of this compound.

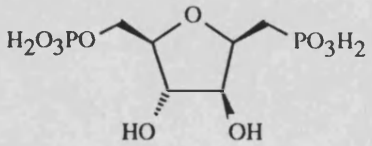
Maryanoff *et al.*[69, 70] investigated the electrophile promoted cyclisation of a hydroxyolefin derived from D-arabinose. The Wittig reaction of 2,3,5-tri-O-benzyl D-arabinose (180) with methylidenephenyl phosphorane gave the hydroxy olefin (181). Treatment of (181) with NBS afforded, almost quantitatively, an inseparable 11:89 mixture of the α- and β-anomers (182) and (183) respectively (**Scheme 30**).



(186)

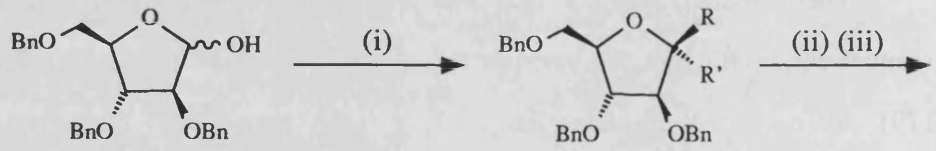


(187) (188)



(189)

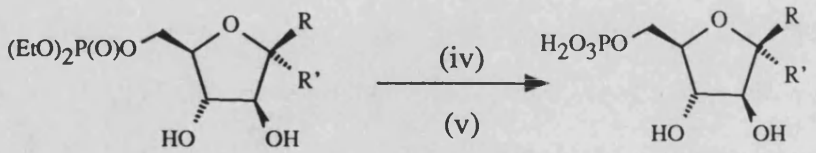
Scheme 31. *Reagents and conditions:* (i) 1% H_2SO_4, Ac_2O, rt, 1h (86%); (ii) aq. NaOH, MeOH, 35°, 5min (92%); (iii) $(PhO)_2P(O)Cl$, Py, rt, 45min (67%); (iv) $(PhO)_2P(OEt)$, 178°, 16h (39%); (v) 10% Bu_4NOH, THF, rt, 24h; (vi) H_2, 10% Pd/C, EtOH, H_2O, rt, 24h; (vii) H_2, PtO_2, rt, 24h (34%).



(180)

(190) R = H, R' = $CH_2(O)P(OEt)_2$

(191) R = $CH_2(O)P(OEt)_2$, R' = H



(192) R = H, R' = $CH_2(O)P(OEt)_2$

(193) R = $CH_2(O)P(OEt)_2$, R' = H

(194) R = H, R' = $CH_2PO_3H_2$

(189) R = $CH_2PO_3H_2$, R' = H

Scheme 32. *Reagents and conditions:* (i) $(EtO)_2P(O)CH_2(O)P(OEt)_2$, NaH, DME, 40°, 4h (85%); (ii) H_2, 10% Pd/C, EtOH, rt (100%); (iii) $(EtO)_2P(O)Cl$, Py, 0° - rt, 12h; (iv) TMSBr, CH_3CN, 0° - rt, 6h; (v) H_2O.

A similar cyclisation of (181) with mercuric acetate produced the mercurials (184) and (185) (after exchange to the bromide) again as an 11:89 mixture. The preponderance of 2,3-cis products on electrophile promoted cyclisations has been attributed to through space co-ordination between the proximal benzyl ether and the electrophile[71, 72].

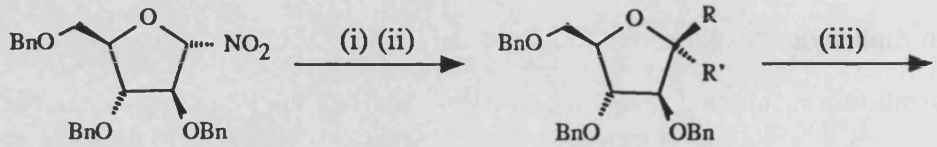
Acid catalysed acetolysis of the 11:89 mixture of (182) and (183) produced the monoacetate (186). Deacylation followed by phosphorylation gave the primary phosphate (187). The phosphonate group was introduced via an Arbuzov reaction with diphenylethyl phosphite to afford the diphenyl phosphonate (188) as a solid. Crystallisation supplied material consisting solely of the β-anomer. Removal of the protecting groups then gave the isosteric phosphonate analogue (189) of β-D-arabinose 1,5-bisphosphate (**Scheme 31**).

Phosphonate analogue (189) was a competitive inhibitor of FBPase with a K_i value of 173μM and for the activation of PFK, the concentration for half maximal activity was 31μM.

McClard *et al.*[73] utilised the Horner-Emmons reaction of (180) with the anion derived from tetraethyl methylenediphosphonate to give the phosphonates (190) and (191) in an α-to-β ratio of 2:1.

The mixture of (190) and (191) was debenzylated and selectively phosphorylated at the 6-position to give a mixture of (192) and (193). Deprotection afforded the isosteric analogue of arabinose 1,5-bisphosphate as a 2:1 α/β anomeric mixture, compared with Maryanoff's synthesis of the single β-anomer (**Scheme 32**)[69, 70].

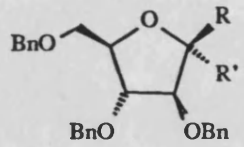
The anomeric mixture inhibits FBPase with a K_i value of 6μM. Hence, the mixture of (189) and (194) was a more effective inhibitor of FBPase than the β-anomer (189) alone. This suggests that the α-anomer (194) may be a potent inhibitor of FBPase, a surprising result as the active form of fructose 2,6-bisphosphate has the β-configuration. It should though be noted that (194) is a



(101)

(195) R = NO_2, R' = $CH_2OCH_2OCH_3$

(196) R = $CH_2OCH_2OCH_3$, R' = NO_2



(197) R = $CH_2OCH_2OCH_3$, R' = CH_2NO_2

(198) R = CH_2NO_2, R' = $CH_2OCH_2OCH_3$

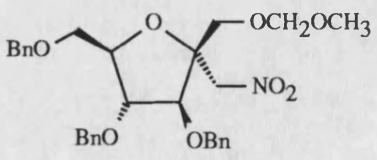
Scheme 33. *Reagents and conditions:* (i) $(CH_2O)_n$, Bu_4NF, CH_2Cl_2, rt, 4h; (ii) $CH_2(OCH_3)_2$, P_2O_5, THF, rt, 6h (66%); (iii) NaH, CH_3NO_2, DMSO, hv, 6h, rt (68%)

non-isosteric analogue of fructose 1,6-bisphosphate, the natural substrate of FBPase, lacking the anomeric hydroxy group.

Meuwly and Vasella synthesised isosteric monophosphonate analogues of α- and β-D-fructose-2,6-bisphosphate[74] from readily available 1-deoxy-1-nitro-D-arabinose (101). Base catalysed Henry reaction of (101) with paraformaldehyde followed by protection of the resulting hydroxy group gave a 4:1 mixture of the methoxy methylethers (195) and (196), respectively. A radical chain substitution of the mixture by nitromethane anion then gave the key intermediates (197) and (198) in a 3:1 ratio (**Scheme 33**).

The nitro compound (198) was converted into the aldehyde (199) by ozonolysis of the nitronate anion. Abramov reaction of (199) with diphenyl phosphite in the presence of triethylamine followed by treatment of the resulting α-hydroxy phosphonates with N,N'-thiocarbonyldiimidazole gave (200) as ca. 9:1 mixture of diastereomers. Barton deoxygenation[75] of the imidazoylthiocarbonyloxy derivatives with tributyltin hydride gave the phosphonate (201). Selective hydrogenolysis of (201) followed by phosphorylation with diphenyl phosphorochloridate in pyridine afforded the primary phosphate (202). Compound (202) was then simply deprotected to give the isosteric mono-phosphonate analogue (203) of β-D-fructose 2,6-bisphosphate (**Scheme 34**).

Similarly, the isosteric mono-phosphonate analogue (204) of α-D-fructose-2,6-bisphosphate was synthesised from the nitro compound (197).



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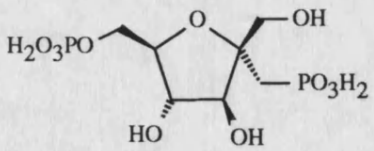
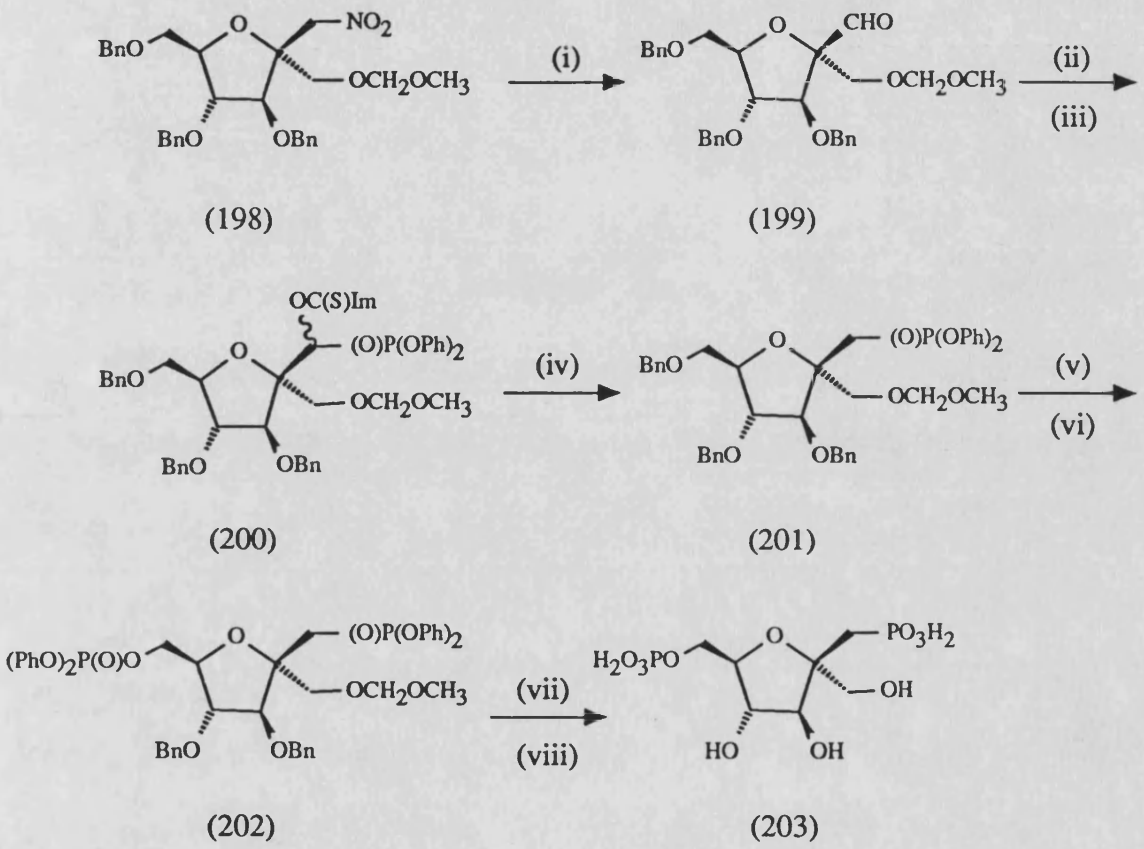
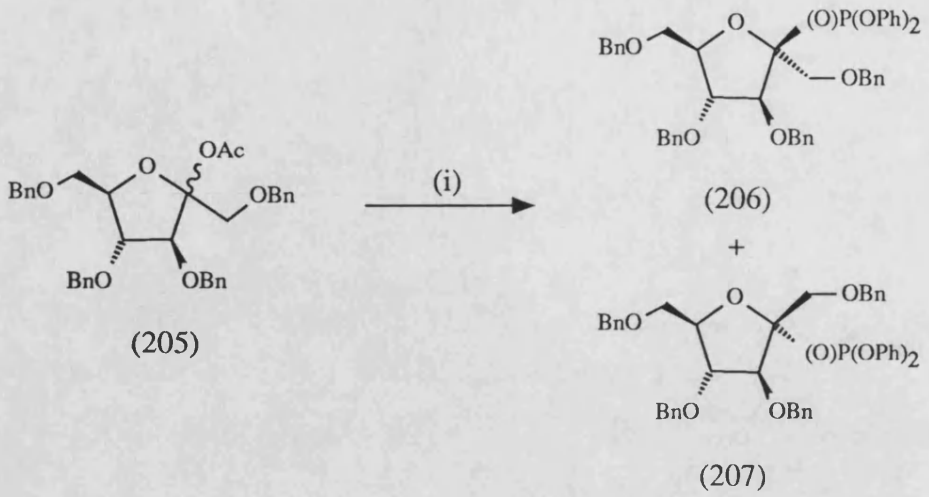


(204)

Unfortunately, the biological activity of these compounds was not reported. However, one would expect one or both of these compounds to be a potent

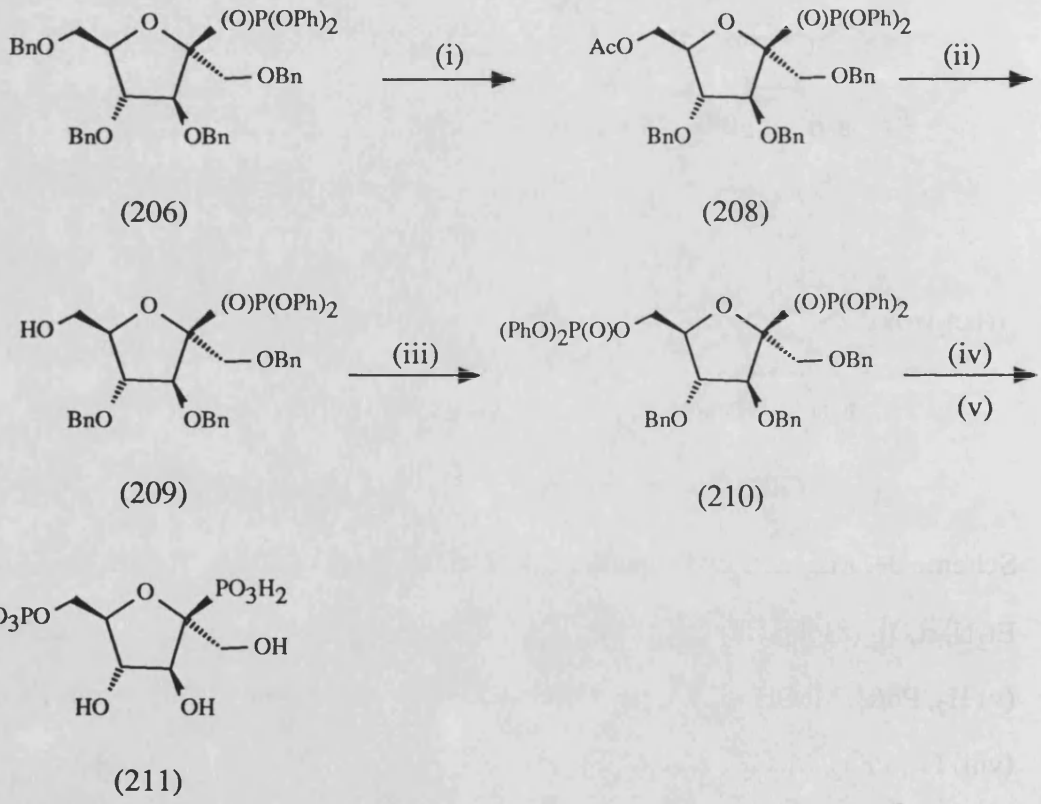


Scheme 34. *Reagents and conditions:* (i) NaOMe, MeOH, O_3, -78° (74%); (ii) $(PhO)_2P(O)H$, Et_3N, rt, 1h (81%); (iii) ImC(S)Im, THF, rt, 4h (85%); (iv) Bu_3SnH, PhMe, reflux, 4h (79%); (v) H_2, Pd/C, MeOH (62%); (vi) $(PhO)_2P(O)Cl$, Py, rt, 30min (91%), (vii) H_2, Pd/C, MeOH; (viii) H_2, PtO_2, MeOH, 24h (66%).



(205) (206) (207)

Scheme 35. *Reagents and conditions:* (i) $(PhO)_3P$, TMSOTf, CH_2Cl_2 (73%).



(206) (208) (209) (210) (211)

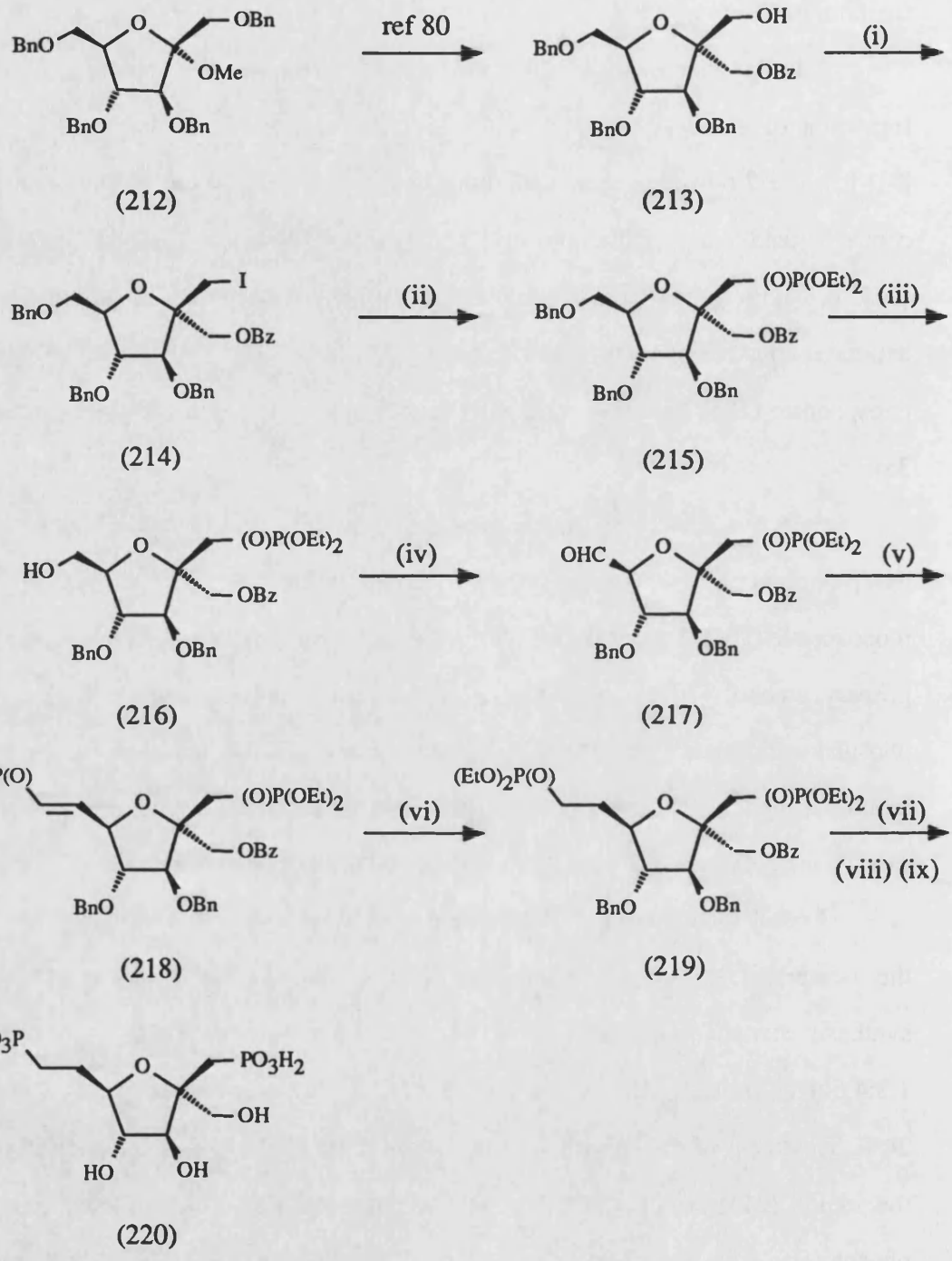
Scheme 36. *Reagents and conditions:* (i) $BF_3.EtO_2$, Ac_2O, -40 - 0°, 6h (67%); (ii) *m*-$NO_2C_6H_4O^-$ Na^+, MeOH, reflux, 3h; (iii) $(PhO)_2P(O)Cl$, Py, 0°, 30min (40%); (iv) H_2, Pd/C, rt, 6h; (v) H_2, PtO_2, rt, 2h (74%).

inhibitor of FBPase.

Vasella also exploited the previously developed methodology for the formation of glycosyl phosphonates[41] to synthesise the 'isopolar' analogue of β-D-fructose 2,6-bisphosphate containing the phosphonate group at the anomeric centre[76]. Readily available 2-O-acetyl 1,3,4,6-tetra-O-benzyl α/β-D-fructopyranose (205, α/β ratio 5.5:1)[77] on treatment with triphenyl phosphite and trimethylsilyl trifluoromethanesulphonate gave predominantly the 2,3-cis-configurated anomeric phosphonate (206) in a 67% yield and the α-anomer (207) in a 6% yield (**Scheme 35**).

Selective acetolysis[78] of the least sterically hindered benzyl ether group of the β-phosphonate (206), catalysed by boron trifluoride etherate, yielded the monoacetate (208). Deacetylation with methanolic sodium nitrophenoxide gave the primary alcohol (209) which was phosphorylated on treatment with diphenyl phosphorochloridate in pyridine. Hydrogenolysis of (210) then gave the isopolar mono-phosphonate analogue (211) of β-D-fructose 2,6-bisphosphate (**Scheme 36**). To date the biological activity of this compound has not been reported.

Recently Nicotra *et al.*[79] described a different approach to the synthesis of the isosteric bisphosphonate analogue of β-D-fructose 2,6-bisphosphate. The synthesis started from the C-glycoside (213) itself synthesised from methyl 1,3:4,6-tetra-O-benzyl-D-fructofuranoside (212)[80] in 6 steps in an overall yield of 24%. Treatment of (213) with triphenylphosphine, iodine and imidazole afforded the iodide (214) which underwent an Arbuzov reaction with refluxing triethyl phosphite to give the phosphonate (215). To introduce the second phosphonate group, the benzyl ether protecting group on C-6 was selectively hydrogenolysed. The free hydroxy group of (216) was then oxidised with PCC to afford the aldehyde (217). Wittig reaction of the aldehyde to obtain an α,β-unsaturated phosphonate proved troublesome. The best result was obtained using the Horner-Emmons modification, thus reaction of tetraethyl methylenediphosphonate



Scheme 37. *Reagents and conditions:* (i) I_2, Ph_3P, imidazole, $PhCH_3$, CH_3CN, reflux (95%); (ii) $(EtO)_3P$, reflux (85%); (iii) H_2, Pd/C, MeOH (62%); (iv) PCC, CH_2Cl_2; (v) $(EtO)_2P(O)CH_2(O)P(OEt)_2$, DBU, LiCl, CH_3CN (40%); (vi) H_2, PtO_2 (96%); (vii) TMSBr, CH_2Cl_2; (viii) H_2, Pd/C; (ix) DOWEX 50 (H^+).

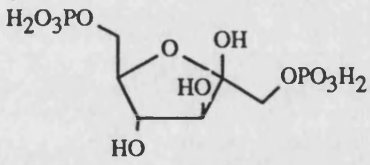
with the aldehyde (217), catalysed with DBU gave a moderate yield of the vinyl phosphonate (218). Compound (218) was hydrogenated on platinum oxide yielding the phosphonate (219) which was deprotected under standard conditions to give the isosteric bisphosphonate analogue (220) of β-D-fructose 2,6-bisphosphate (**Scheme 37**).

Preliminary tests to check the effectiveness of (220) as an activator of PFK and as an inhibitor of FBPase indicated that (220) was ca. three orders of magnitude less effective than β-D-fructose 2,6-bisphosphate. Thus, the replacement of the oxygen atoms of the phosphate ester linkages with methylene groups dramatically decreases the affinity of the molecule for the binding sites of the aforementioned enzymes.

RESULTS AND DISCUSSION

Aims and Objectives

The primary objective was to synthesise phosphonate analogues of fructose phosphates in which the phosphate group was directly replaced by the phosphonate moiety.



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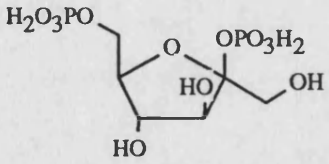


(167)

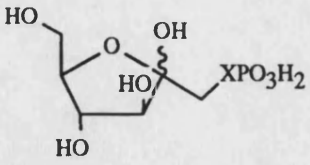
The distinctive enzyme involved in gluconeogenesis is fructose 1,6-bisphosphatase (FBPase) which catalyses the hydrolysis of fructose 1,6-bisphosphate (166) to fructose 6-phosphate. As the control element of gluconeogenesis FBPase plays an essential role in controlling blood sugar levels.

If the 1-phosphate group was replaced by a non-hydrolysable phosphonate group, the resulting molecule may still be recognised as a substrate by FBPase. This analogue would then act as a competitive inhibitor of FBPase by occupation of the active site.

Hence, the initial aim was to synthesise the non-isosteric phosphonate analogue (224) of fructose 1-phosphate (223).

Other possible targets are the thiophosphate (225), in which the O atom of the phosphate group is replaced with a S atom, and the phosphoroamidate (226), in which the O is replaced by NH. Although thiophosphate and phosphoroamidate analogues of phosphates have been shown to be recognised by enzymes both have the disadvantage that they are readily hydrolysed.

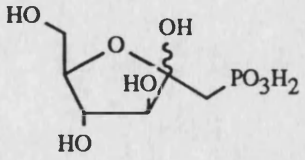
Also of interest would be analogues of fructose 2,6-bisphosphate (167), in



(223) X=O

(225) X=S

(226) X=NH

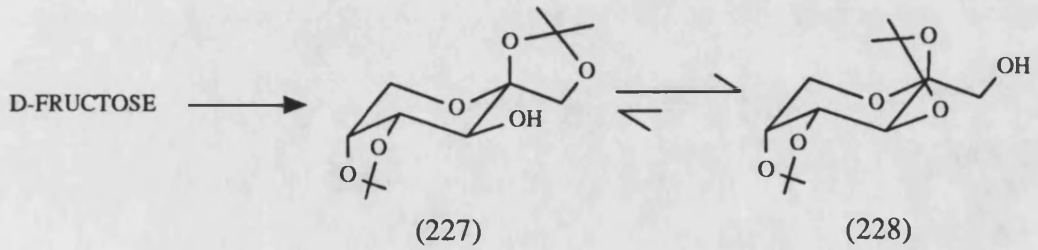


(224)

which the readily enzymically and chemically hydrolysable anomeric phosphate group has been replaced by a stable phosphonate group. Such an analogue may retain the same properties as the parent molecule in that it would stimulate phosphofructokinase (PFK) and inhibit FBPase

Synthesis and Attempted Michaelis-Arbuzov Reaction of Primary Halides.

The standard method for the synthesis of phosphonates is *via* either the Michaelis-Arbuzov[81] or Michaelis-Becker[82] reactions on the corresponding alkyl iodide or bromide. The first problem to be addressed was to differentiate between the five hydroxyl groups of D-fructose. This is most conveniently achieved by exploiting the reaction of D-fructose with acetone under acidic catalysis. Condensation of D-fructose with acetone in the presence of sulphuric acid gives initially 1,2;4,5-di-O-isopropylidene-β-D-fructopyranose (227). This compound isomerises at a rate dependent on the concentration of the acid to 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (228)[83].



(227) (228)

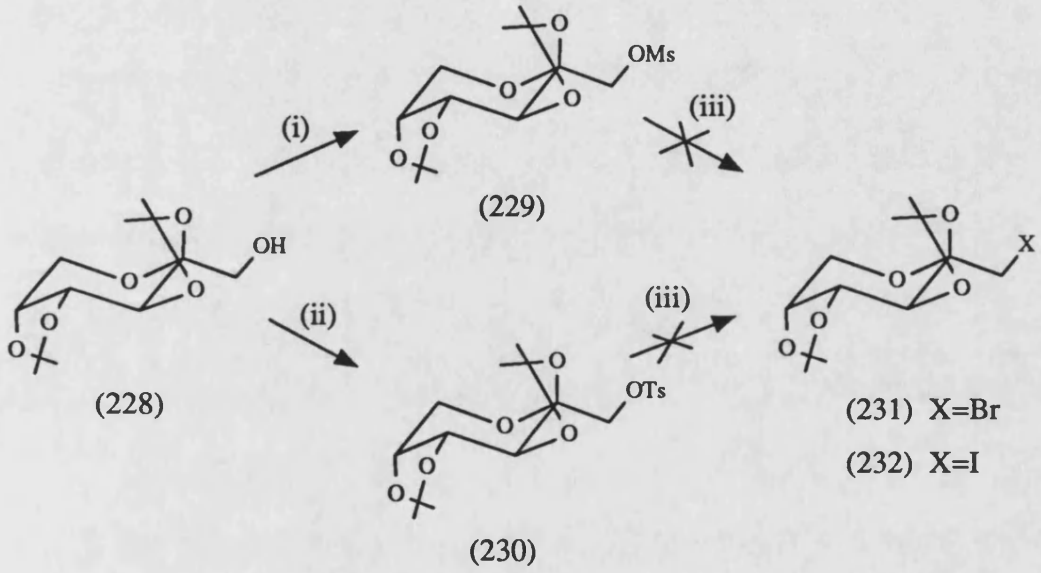
Figure 8.

Diacetonide (227), the kinetic product of the reaction, is formed first presumably as a consequence of the higher reactivity of the primary hydroxyl group on C-1. Diacetonide (228) greatly preponderates at equilibrium. Thus, it is thermodynamically more stable than (227) (**Figure 8**). At low concentrations of catalyst (≤0.5%), (227) slowly isomerises to (228), but if the reaction is arrested before attainment of equilibrium between (227) and (228), compound (227) may be isolated in satisfactory yield. At high concentrations of catalyst (≥5%), (227) is formed first but isomerises rapidly to (228).

In acetone containing 5% sulphuric acid, the rearrangement of (227) to (228) has been found to be rapid at room temperature; within 5 min, each pure

isomer being converted into an equilibrium mixture consisting of 94% of (228) and 6% of (227)[83].

Thus utilising a high concentration of catalyst (5%) and allowing the reaction to reach equilibrium a 70% recrystallised yield of 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (228) was obtained. This gave direct access to the primary hydroxyl group on C-1, and the requirement now was to transform the hydroxyl group into the corresponding iodide or bromide.



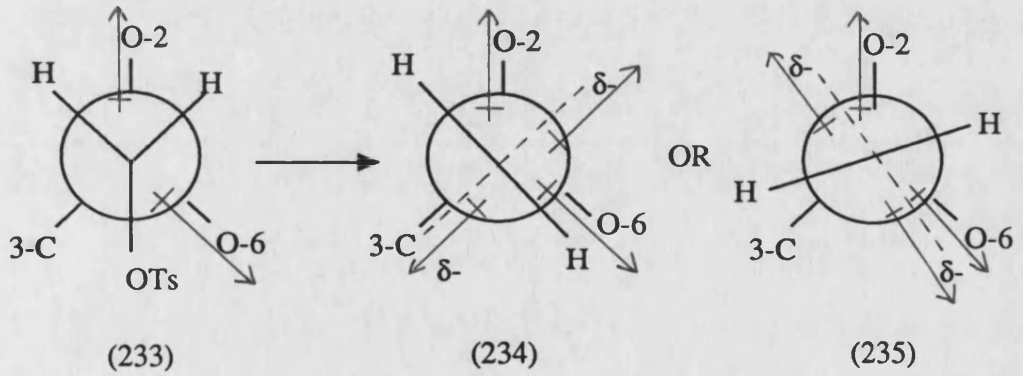
Scheme 38. *Reagents and conditions:* (i) MsCl, Py, -40°- rt, 2h (78%); (ii) TsCl, Py, rt, 24h (76%); (iii) KX, DMF, 150°, 12h

Barnett and Atkins[84] synthesised the 1-chloro-1-deoxy and 1-bromo-1-deoxy derivatives from the 1-O-methanesulphonyl-2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (229) in moderate and poor yields respectively, by reaction with LiX (X=Cl, Br) in DMF at 144°C[84]. However, in our hands reaction of the mesylate (229)[84] or tosylate (230)[85] with either potassium bromide or potassium iodide in DMF at 150° did not furnish the required primary halides (**Scheme 38**).

Richardson[86] attributed the resistance to nucleophilic attack by ionic

reagents of hexulopyranose 1-sulphonates to the dipolar interaction of the ring and anomeric oxygen bonds with the transition state dipole.

The displacement of a sulphonyl group normally takes place by an S_N2 mechanism. The formation of the S_N2 transition state involves the generation of two highly polar bonds, one in the process of formation and the other in the process of degeneration. The formation of these polar bonds will be greatly affected by the presence of neighbouring polar substituents, and if they are electronegative in character, the permanent dipole associated with the substituent to carbon bond hinders the development of the transition state when an anionic nucleophile is used. This effect will be maximal when the dipoles are antiparallel.



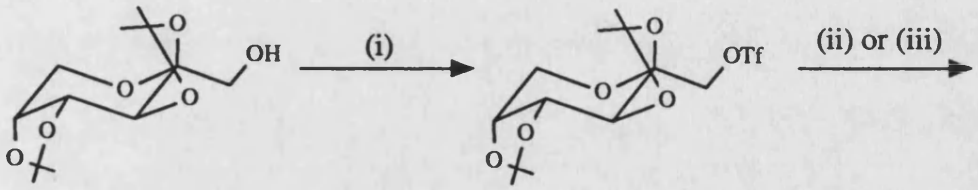
(233) (234) (235)

Figure 9.

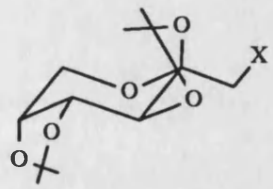
In the case of 1-O-*p*-toluenesulfonyl-2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (230) the permanent dipoles about the anomeric centre are best visualised by projection along the C-1 - C-2 bond (233). The permanent dipoles give rise to considerable dipolar repulsions in the two extreme transition states (234) and (235) hence the formation of either would be unfavourable (**Figure 9**). In addition to this electronic effect, C-1 is neopentyl in nature so the rate of S_N2 reaction would be very slow due to steric hindrance. The unreactivity of the

mesylate (229) and tosylate (230) can be overcome by recourse to a better leaving group. The trifluoromethanesulfonate (triflate) leaving group is some 10^4 to 10^5 times more reactive than the corresponding tosylates[87].

Binkley *et al.* have utilised the unrivalled leaving group ability of triflate esters to synthesise a variety of deoxy-iodo sugars, by reaction of the triflate with tetrabutylammonium iodide in refluxing benzene[88]. The primary triflate (236) was prepared *via* Binkley's procedure, and readily reacted with either potassium bromide or potassium iodide in DMF at 70° to furnish the bromide (231) and the iodide (232) respectively, in high yield (**Scheme 39**).



(228) (236)



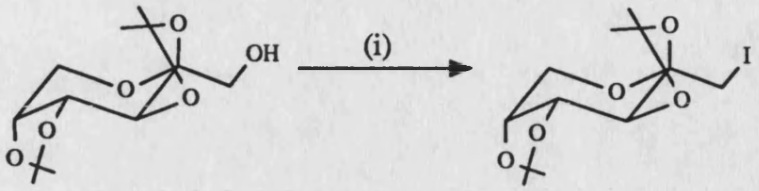
(231) X=Br

(232) X=I

Scheme 39. *Reagents and conditions:* (i) Tf_2O, Py, CH_2Cl_2, -15°, 90min (95%); (ii) KBr, DMF, 70°, 18h (89%); (iii) KI, DMF, 70°, 18h (96%).

However, the most convenient route to the primary iodide was directly from the alcohol (228) using the phosphorus based reagent developed by Garegy and Samuelsson[89]. Treatment of the primary alcohol(228) with triphenylphosphine, imidazole and iodine in refluxing toluene gave the iodide (232) directly in 98%

yield (**Scheme 40**).



(228) (232)

Scheme 40. *Reagents and conditions:* (i) I_2, Ph_3P, Im, PhMe, reflux, 18h (98%).

The mechanism postulated for the transformation was that illustrated in **Figure 10**.

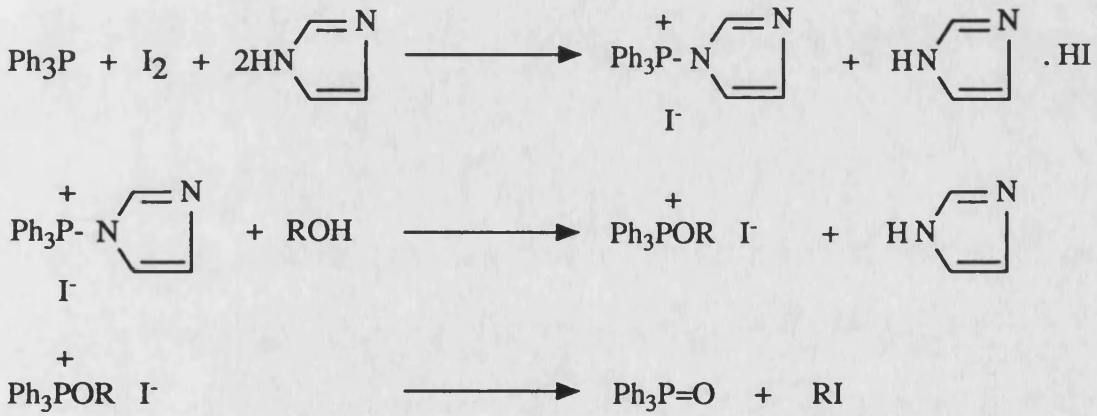
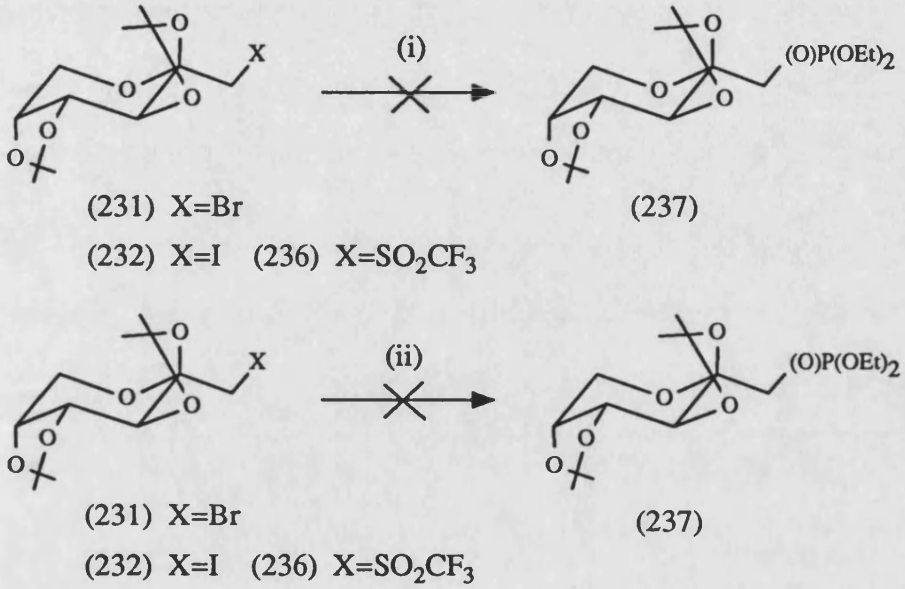


Figure 10.

In the absence of imidazole, triphenylphosphine and iodine in toluene form an adduct which is virtually insoluble. The driving force for the reaction is the formation of a strong P=O bond, and since the leaving group in this case is neutral, it does not give rise to unfavourable interactions with the neighbouring electronegative substituents in the transition state[86].

The 1-bromo and 1-iododeoxy sugars were now readily available.

Unfortunately, neither underwent Michaelis-Arbuzov or Michaelis-Becker reaction under any of the conditions attempted. Thus, attempted Michaelis-Arbuzov reaction of (231) or (232), initially with triethyl phosphite at reflux and then at 200° in a sealed tube for prolonged periods, yielded only starting material and non-phosphorus containing decomposition products.

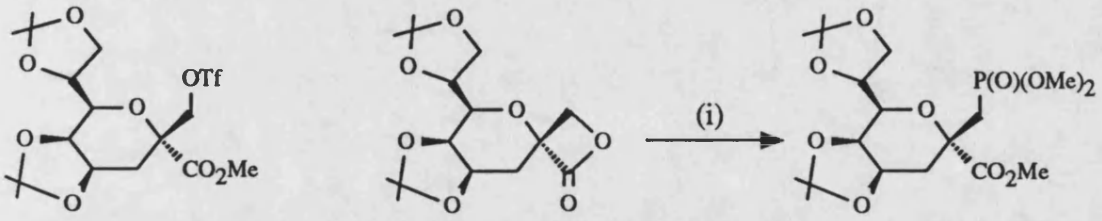


Scheme 41. *Reagents and conditions:* (i) $(EtO)_3P$, reflux; (ii) $NaP(O)(OEt)_2$, PhMe, reflux

Similarly, reaction of (231) or (232) with sodium diethyl phosphite in refluxing toluene gave only starting material (**Scheme 41**). The failure to form the required phosphonate is almost certainly again due to the difficulty in accomplishing S_N2 displacements at C-1 of this particular system.

Triflate esters are known to undergo the Michaelis-Arbuzov reaction[90], but even the more reactive triflate ester (236) failed to give the required primary phosphonate (237) on reaction with refluxing triethyl phosphite or sodium diethyl phosphite in refluxing toluene (**Scheme 41**). The failure to displace a triflate ester by a phosphorus nucleophile has been reported by Norbeck *et al.*[45] in a similar

system (**Scheme 42**). The triflate ester (138) was inert to refluxing triethyl phosphite. However, utilisation of the carboxyl residue as a leaving group divested the S_N2 transition state of its neopentyl character. Thus, refluxing the β-lactone (139) in trimethyl phosphite gave the phosphonate (140) albeit in low yield[45].



(138) (139) (140)

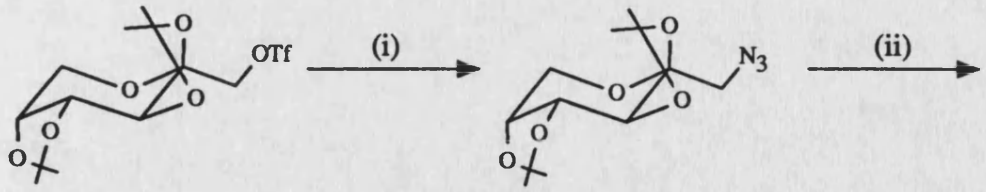
Scheme 42. *Reagents and conditions:* (i) $(MeO)_3P$, reflux, 24h (37%)

Although the triflate (236) was inert to phosphorus nucleophiles, it was readily displaced by nitrogen and sulphur nucleophiles, affording compounds which could be readily modified to give phosphoroamidates and thiophosphates.

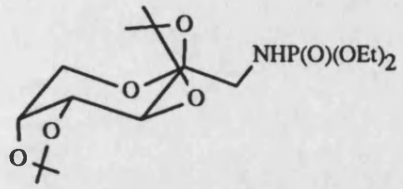
Card *et al.*[91] reported the synthesis of the primary azide (238) on treatment of the triflate (236) with a saturated sodium azide solution in DMF at 80°C for 18h. In contrast to this report, it was found that reaction of the triflate (236) with 1 equiv. of sodium azide was complete after 12h at 70° to give the azide (238) in quantitative yield. The reason for this slight discrepancy was probably due to the fact that the triflate and the azide co-elute on thin layer chromatography using most solvent systems.

Staudinger reaction[92] of the azide (238) with triethyl phosphite in toluene then afforded cleanly the diethyl phosphoroamidate (239) (**Scheme 43**).

Coincidentally with our attempts to synthesise the primary thiol (244), Goodwin[93] described the nucleophilic displacement of the triflate in (236) by potassium thiolacetate to give 1-S-acetyl-2,3:4,5-di-O-isopropylidene-thio-



(236) (238)

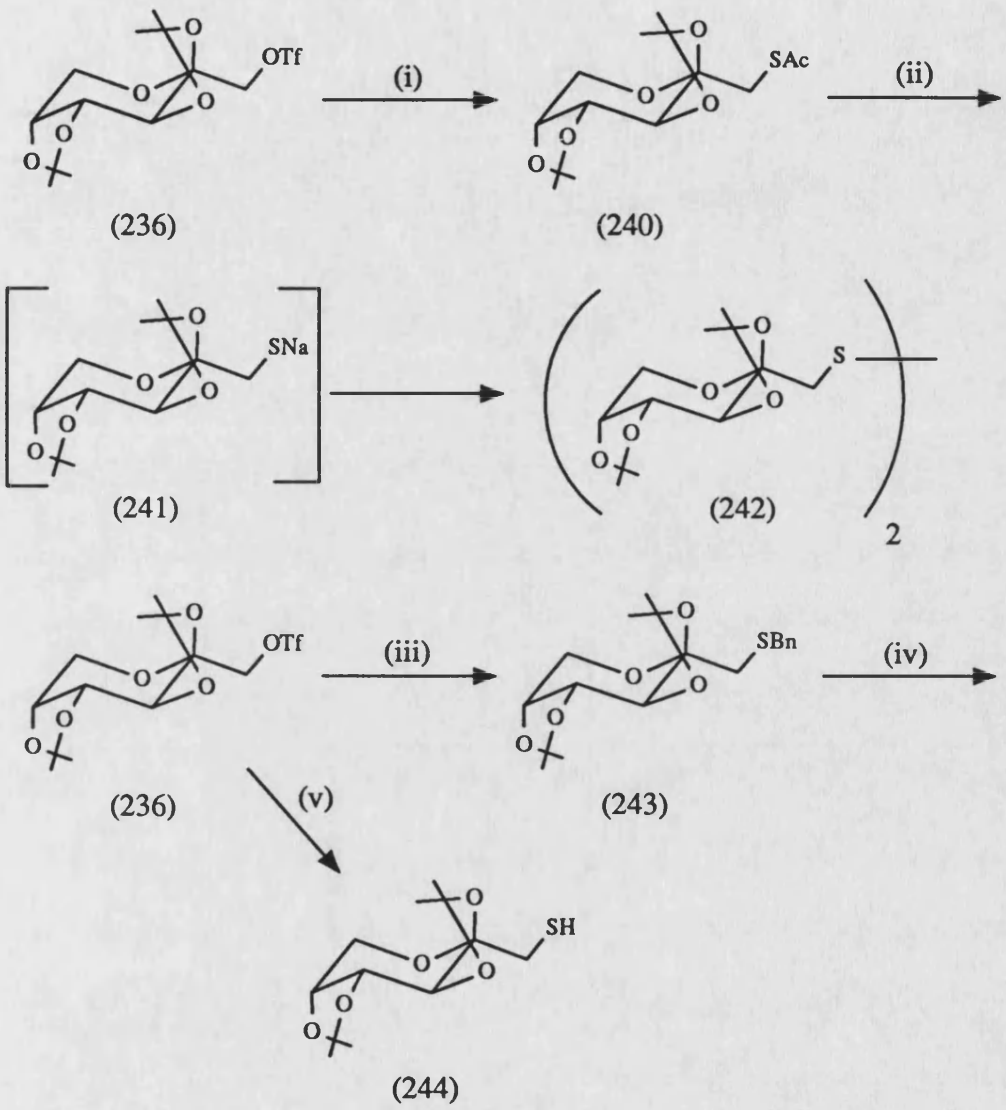


(239)

Scheme 43. *Reagents and conditions:* (i) NaN_3, DMF, 70°, 12h (100%); (ii) $(EtO)_3P$, PhMe, 70°, 3h (92%)

β-D-fructopyranose (240). Basic hydrolysis with NaOMe yielded the sodium thiolate salt (241) which was reported to be unstable and readily underwent oxidative dimerisation[94] whereby a disulphide dimer (242) was formed (**Scheme 44**). Mercaptans are readily oxidised on standing, by the oxygen in the atmosphere, if a small amount of base is present.

However, the thiol (244) was readily prepared using a different method of approach. Reaction of the triflate (236) with sodium benzylmercaptan[95] in DMF afforded the benzylsulphide (243) in excellent yield. The benzyl protecting group was readily removed on treatment with sodium in liquid ammonia[96] to afford the thiol (244). Thiol (244) could also be prepared directly from the triflate (236) on treatment with sodium hydrosulphide hydrate[97] in DMF at 80°C. Under neither sets of the conditions used for the preparation of the thiol (244), was any formation of the disulphide (242) observed. The ^1H n.m.r. spectrum of (244) contained a doublet of doublets at 1.70ppm corresponding to the thiol proton which is coupled



Scheme 44. *Reagents and conditions:* (i) KSAc, acetone, rt, 20h (76%); (iii) NaOMe, MeOH, rt, 5min; (iii) NaSBn, DMF, rt, 18h (96%); (iv) Na, liq. NH_3, 30min (72%); (v) $NaSH.xH_2O$, DMF, 80°, 18h (73%)

to both C-1 methylene protons. The resonances of the C-1 protons at 2.72 and 3.07ppm also appeared as doublet of doublets with coupling constants to the thiol proton of 10.6 and 7.0Hz respectively. On D_2O shake the resonance at 1.70ppm disappeared and the resonances due to the C-1 protons were simplified to doublets.

The thiol (244) was stable at room temperature, with no decomposition seen to occur over a prolonged period of time.

A simple reaction of the thiol (244) with a dialkyl chlorophosphate should afford a protected thiophosphate although this was not investigated.

Although nucleophilic displacement of the triflate (236) was a viable route for the formation of thiophosphate and phosphoroamidate analogues of D-fructose 1-phosphate, the lack of success in displacing the triflate ester with phosphorus nucleophiles necessitated a different approach for the synthesis of a primary phosphonate.

Synthesis of an Anomeric Spiro-epoxide

Epoxides undergo facile ring-opening with a large variety of nucleophiles, the relief of ring strain providing a potent driving force for the reaction.

The base catalysed ring-opening of epoxides is subject to steric influences and occurs at the least hindered carbon atom, since this is the position most open to nucleophilic attack[98] **(Figure 11)**.

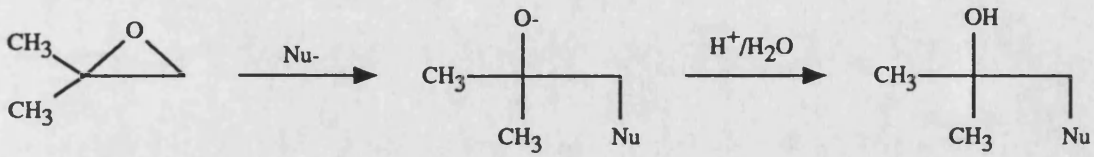


Figure 11.

In contrast the acid catalysed reaction is essentially a carbocation reaction, and reaction tends to occur at the ring carbon which corresponds to the more stable carbocation[99] **(Figure 12)**.

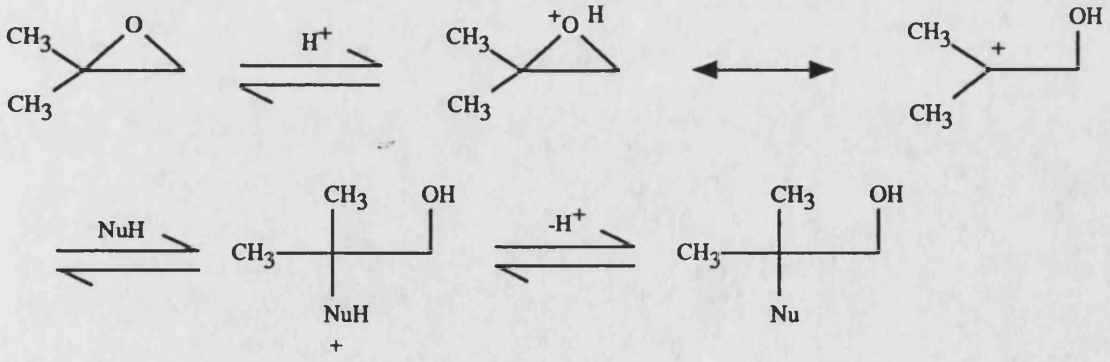
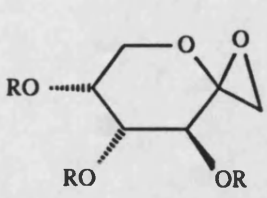
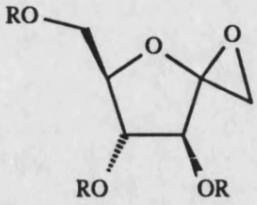


Figure 12.

Hence, a new class of carbohydrate compounds, anomeric spiro-epoxides (245) and (246), were the targets of synthesis.

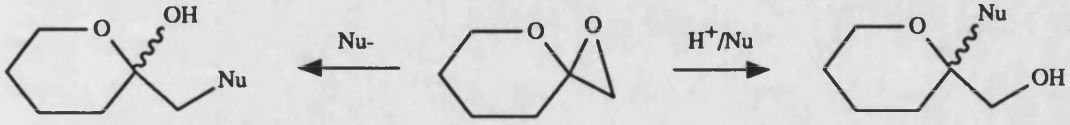


(245)



(246)

Reaction under basic conditions should occur at the least hindered carbon atom, C-1, and lead to analogues of fructose 1-phosphate (247). Conversely, the acid catalysed ring-opening would be expected to occur at the anomeric centre, the most stable carbocation, and lead to analogues of fructose 2-phosphate (248) (**Figure 13**).

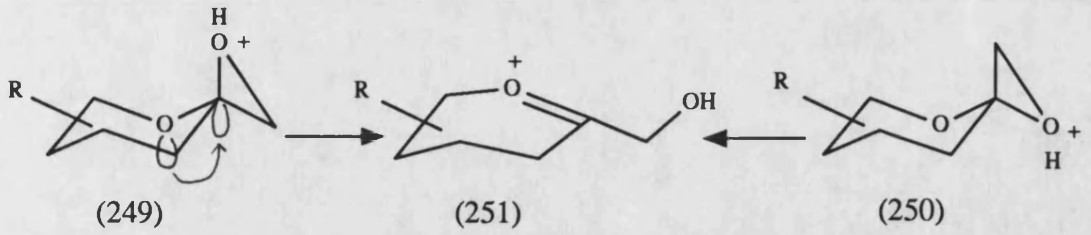


(247) (248)

Figure 13.

Due to the kinetic anomeric effect[100] the anomeric epoxide with an axial C-O bond may cleave quicker under acid catalysis than the epoxide with the equatorial C-O bond.

The epoxide (249) can cleave smoothly, maintaining maximal n-σ^*overlap throughout the process. Whereas if epoxide (250) is to cleave it must do so without assistance from the lone pairs on the ring oxygen atom (**Figure 14**). However, this does not apply if (250) can ring-flip to allow cleavage of an axial C-O bond. In this case, the epoxide with the equatorial C-O bond would be likely to react more rapidly. This is because both epoxides cleave to give the same oxocarbonium ion (251), and thus *via* transition states of very similar energy, their relative reactivity

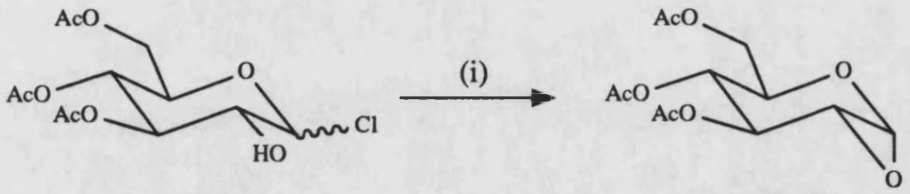


(249) (251) (250)

Figure 14.

would be determined largely by their ground state energies.

Sugar derivatives having an anomeric epoxide fused to the ring are well known in the literature, but have received little interest until recently. In most cases the synthesis of these compounds was achieved by substitution of a leaving group at the anomeric centre by a hydroxyl group on the adjacent carbon atom. The first reported anomeric epoxide was 1,2-anhydro-3,4,6-tri-O-acetyl-α-D-glucopyranose (Brigl's anhydride) (253) as early as 1922. The key step of the synthesis was the base catalysed displacement of chloride by the hydroxyl group on C-2 of the glucopyranosyl chloride (252) to give the anomeric epoxide (253) in zero to moderate yields[101] (**Scheme 45**).

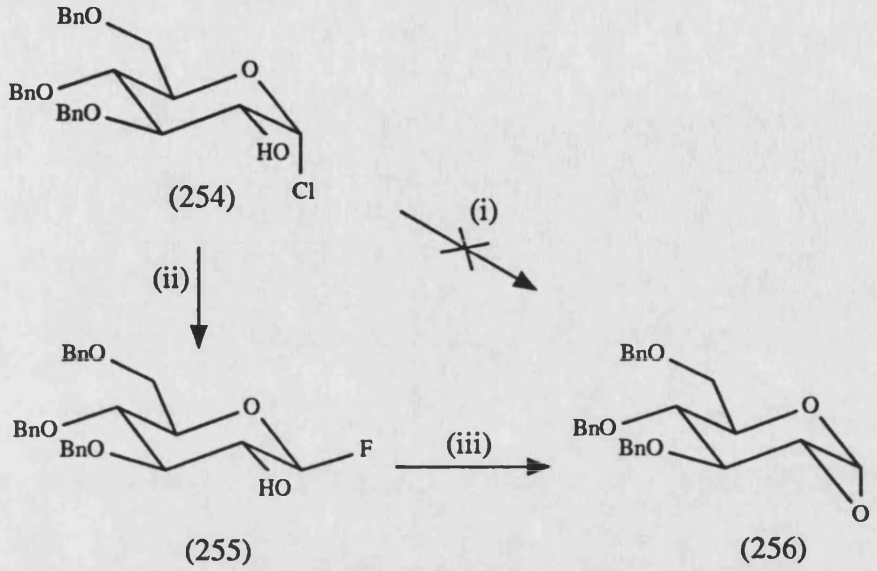


(252) (253)

Scheme 45. *Reagents and conditions:* (i) $NH_3(g)$, PhH (0-55%)

It did not prove beneficial to start with the pure β-anomer of (252) as it immediately anomerised on contact with the reagents used for the ring closure[102].

Yamaguchi and Scherch, very much later, synthesised



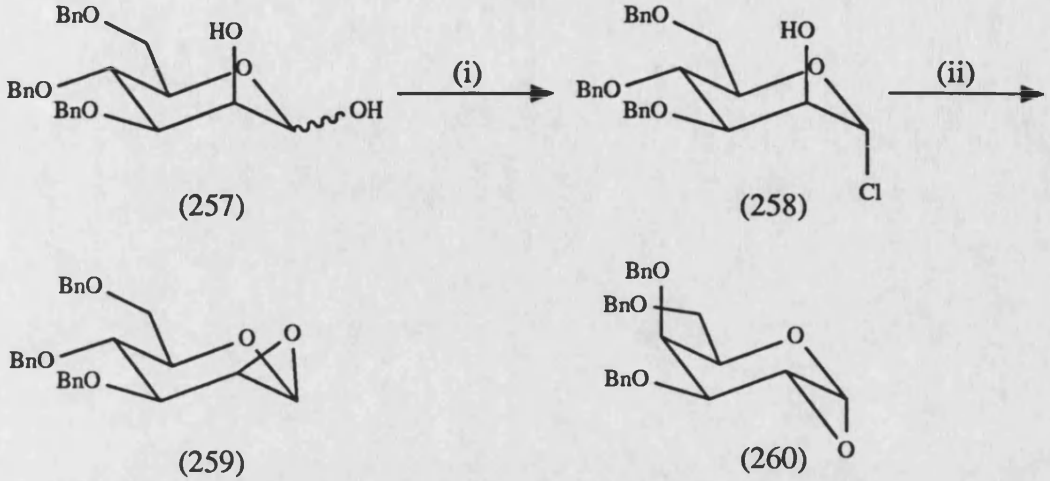
Scheme 46. *Reagents and conditions:* (i) $NH_3(g)$, PhH; (ii) AgF, PhH, CH_3CN, rt, 18h (40%); (iii) K^+ *t*-BuO^-, rt, 5h (34% from (254)).

1,2-anhydro-glucopyranose with benzyl protecting groups[103].

3,4,6-Tri-O-benzyl- α-D-glucopyranosyl chloride (254) failed to react with ammonia and could be recovered largely unchanged from the reaction mixture, presumably because anomerisation does not take place. However, modification of the Micheel and Kreutzer[104] method for the formation of 1,4-anhydro-α-D-glucopyranose proved successful. Reaction of (254) with silver fluoride gave the β-D-glucopyranosyl fluoride (255). Ring-closure was effected on treatment of (255) with potassium *tert*-butoxide in benzene to give the anomeric epoxide (256) (**Scheme 46**).

Similar methodology was used by Yamaguchi *et al.* for the synthesis of a 1,2-anhydro-mannose[105].

Treatment of 3,4,6-tri-O-benzyl-D-mannose (257) with a hydrogen chloride saturated etheral solution gave the α-D-mannopyranosyl chloride (258). Ring closure is mechanistically straightforward, as the C-1 and C-2 substituents are in a

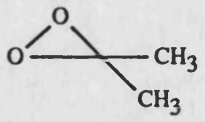


Scheme 47. *Reagents and conditions:* (i) sat. aq. HCl, Et_2O, 0°, 2days; (ii) $NH_3(g)$, PhH, rt, 3h (70% from (257))

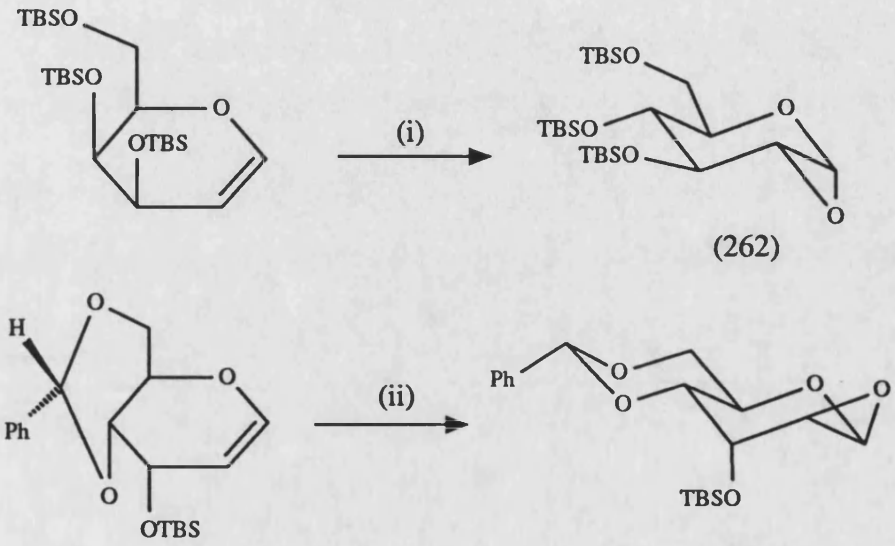
trans diaxial relationship. The use of anhydrous ammonia in benzene, as in the case of Brigl's anhydride (253), afforded cleanly 1,2-anhydro-3,4,6-tri-O-benzyl-β-D-mannopyranose (259) (**Scheme 47**). Also prepared in a similar manner was 1,2-anhydro-3,4,6-tri-O-benzyl-α-D-galactopyranose (260) by Kong *et al.*[106]

Until recently, attempts to synthesise 1,2-anhydro sugars *via* direct epoxidation of glycals had proved unsuccessful. Instead, there were obtained products that corresponded to reaction of the initially formed 1,2-anhydro sugar, with either solvent or acid, RCO_2H, derived from reduction of the peracid RCO_3H[107]. Indeed, Berti *et al.* utilised this well documented experimental result for the facile synthesis of 5-C-alkoxy-D-galactopyranosides[108].

This problem, was overcome by Danishefsky *et al.*[109] using anhydrous 3,3-dimethyl dioxirane[110] (261) as the epoxidant. The by-product, acetone, generated in the reaction, would not then react with the 1,2-anhydro sugar produced.



(261)



(262)

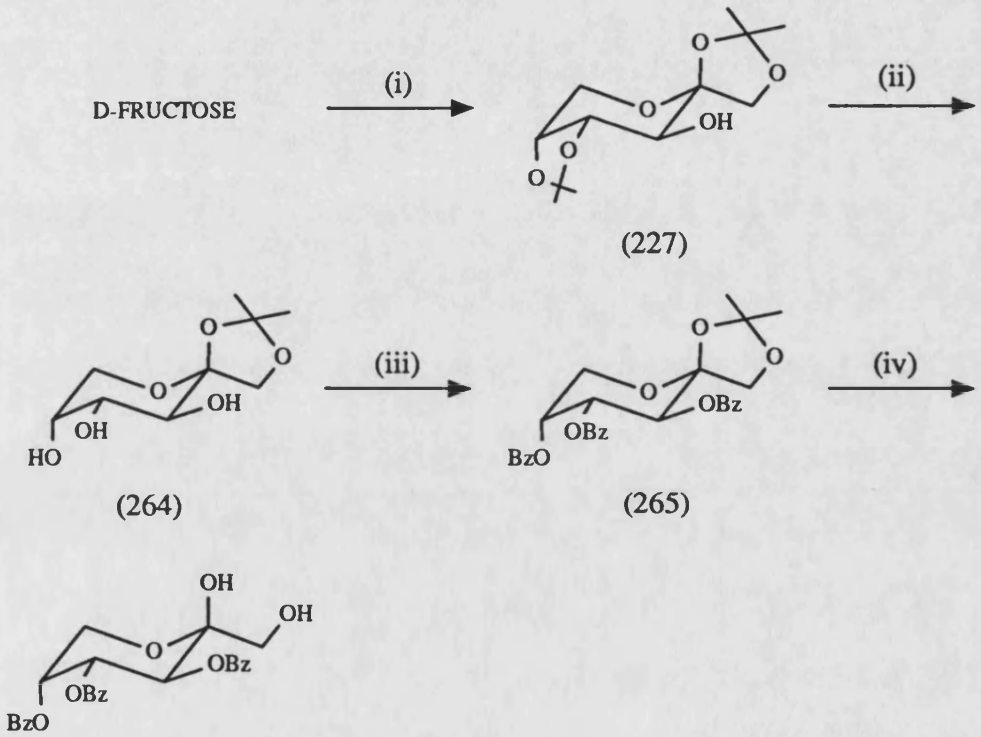
(263)

Scheme 48. *Reagents and conditions:* (i) (261), CH_2Cl_2, 0°, 1h (98%); (ii) (261), CH_2Cl_2, 0°, 1h (98%).

Prepared in this manner were 1,2-anhydro-3,4,6-tri-O-*t*-butyl -dimethylsilyl-α-D-glucopyranose (262) and 1,2-anhydro-4,6-O-benzylidene-3-O-*t*-butyldimethylsilyl-β-D-altropyranose (263) (**Scheme 48**).

However, at the outset of this work anomeric spiro-epoxides had not been reported in the literature. Therefore, it was decided to attempt the formation of a spiro-epoxide by the displacement of a leaving group by an adjacent hydroxyl group . The previous synthesis of anomeric epoxides derived from aldoses had

involved displacement of a leaving group from the anomeric centre. Although this route was viable for ketoses, our synthesis entailed transformation of the most readily accessible primary hydroxyl group to a suitable leaving group, followed by displacement by the anomeric hydroxyl group.

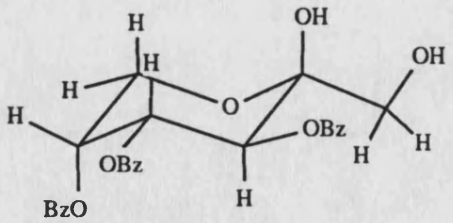


Scheme 49. *Reagents and conditions:* (i) 0.5% v/v H_2SO_4, acetone, rt, 1h (60%); (ii) 0.1% aq. HCl, rt, 24h (82%); (iii) BzCl, Py, 70°, 8h (88%); (iv) 50% aq. TFA, rt, 16h (75%).

The initial problem addressed was to achieve the correct protective pattern, allowing access to the hydroxyl groups on C-1 and C-2 of D-fructose. Again the acid catalysed condensation of D-fructose with acetone[83] was exploited, in this case, using a low concentration of sulphuric acid, 0.5%. Arresting the reaction after 1h, but before attainment of equilibrium between the di-acetonides (227) and

(228), allowed the isolation of 1,2:4,5-di-O-isopropylidene-β-D-fructopyranose (227) in 60% yield. Selective hydrolysis with 0.1% aqueous hydrochloric acid at room temperature for 24h yielded exclusively the triol[111] (264). No hydrolysis of the spiroketal was observed. The resulting triol was then simply protected as the tri-benzoate ester (265) on treatment with 3 equiv. of benzoyl chloride in dry pyridine[112] at 80°C for 4h.

The remaining acetonide protecting group proved resistant to acid catalysed hydrolysis. Treatment with Dowex 50(H^+) in 50% aqueous ethanol at 70°[113], 90% aqueous acetic acid at reflux[114], or 2M hydrochloric acid at reflux[115] yielded only starting material. However, the acetonide was cleanly removed on stirring in 50% aqueous trifluoroacetic acid at room temperature[116] for 18h to yield the diol (266) as a single anomer, with the β-configuration (**Scheme 49**). The anomeric configuration was inferred from the optical rotation which was similar in value to that for the diacetonide (227), the anomeric configuration of which, had been unequivocally established by X-ray crystallography[117]. The diol (266) also had the $^2C_5$ conformation, expected for the β-configuration, indicated by the 3-H, 4-H vicinal coupling constant of 10.5Hz consistent with a diaxial arrangement of these two protons[118] (**Figure 15**).



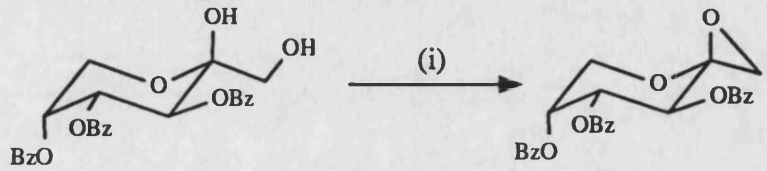
$^3J_{3,4}$ 10.5Hz

(266)

Figure 15.

Direct epoxidation of diols is possible using the Mitsunobu reagents[119], diethyl azodicarboxylate (DEAD), and triphenylphosphine. This was investigated

as a possible route for the synthesis of the anomeric epoxide. Thus, reaction of the diol (266) with 1 equiv. of DEAD and triphenylphosphine in chloroform at room temperature for 18h led to complete consumption of the starting material, giving a complex mixture of products.



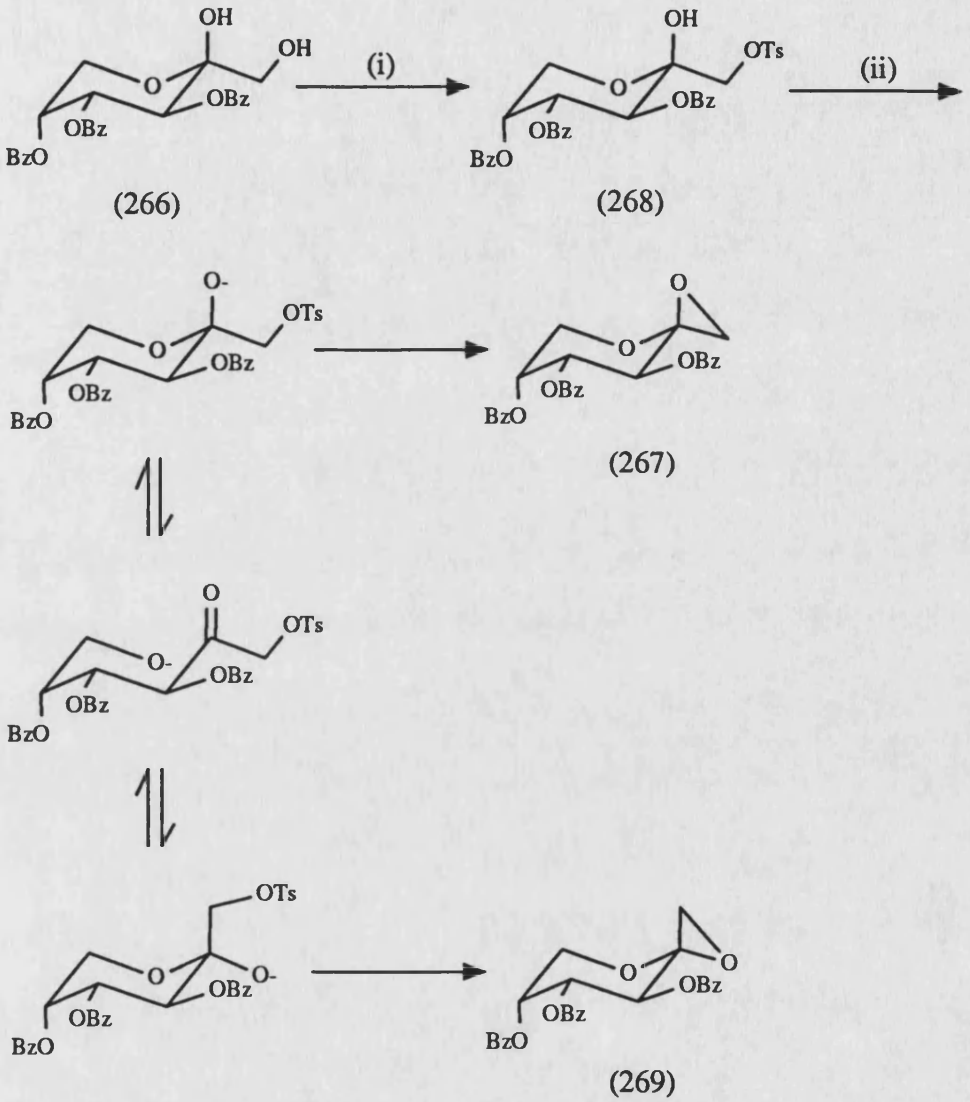
(266) (267)

Scheme 50. *Reagents and conditions:* (i) DEAD, Ph_3P, $CHCl_3$, rt, 18h (0% isolated yield)

N.m.r. of the crude reaction mixture indicated the presence of oxirane protons occurring at high field. In addition, only the β-epoxide appeared to be present (by comparison to the spectra of the anomeric-mixture of epoxides obtained later) (**Scheme 50**). However, assignment of the ^1H n.m.r. spectrum was complicated by the hydrazine by-product formed in the reaction. Attempted flash column chromatography of the reaction mixture on several occasions proved unsuccessful.

Reaction of the diol (266) with 1 equiv. of *p*-toluenesulphonyl chloride in dry pyridine afforded exclusively the primary tosylate (268). Deprotonation of the anomeric hydroxyl group with a non-nucleophilic base should lead to an intramolecular S_N2 displacement yielding the required anomeric spiro-epoxide. On treatment with one equivalent of sodium hydride in dry THF at room temperature for 1h, the tosylate (268) was quantitatively converted to the epoxide. However, in competition with tosylate displacement was reversible tetrahydropyran ring-opening, leading to the formation of an anomeric mixture of spiro-epoxides (267) and (269) (**Scheme 51**).

The α:β ratio, calculated from the integration of the C-1 protons, was 3:4.



Scheme 51. *Reagents and conditions:* (i) TsCl, Py, rt, 48h (96%); (ii) NaH, THF, rt, 1h (85%).

The use of potassium *tert*-butoxide as a base afforded a similar anomeric ratio.

The expected upfield shift of the C-1 methylene protons was observed for both anomers, to give an AB system at 3.06ppm for the α-anomer, and a pair of doublets at 2.90 and 3.14ppm for the β-anomer. In addition the geminal coupling constant was considerably reduced to -4.8 and -3.9Hz, for the α- and β-anomers

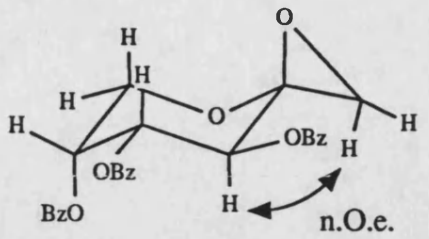
respectively, values consistent with oxirane methylene protons[118]. The C-1 and C-2 signals in the ^{13}C n.m.r. spectrum were similarly shifted to higher field (**Table 1**).

Compound	C-2	C-1 (ppm)
3,4,5-Tri-O-benzoyl-1-O-*p*-toluenesulphonyl -β-D-fructopyranose (268)	95.98	70.45
1,2-anhydro-3,4,5-tri-O-benzoyl -α-D-fructopyranose (269)	81.15	50.37
1,2-anhydro-3,4,5-tri-O-benzoyl -β-D-fructopyranose (267)	82.61	50.37

Table 1

Although the anomers were separable by t.l.c., the R_F values of the α- and β-anomers being 0.64 and 0.69 (60:40 v/v light petroleum:ethyl acetate) respectively, separation by standard techniques proved impossible. The epoxides decomposed on attempted flash column chromatography presumably to the diol (266). The epoxides had a limited lifetime on neutral alumina plates using a chromatatron. However, even using this technique the epoxides quickly decomposed, which necessitated quick elution from the alumina plate and in consequence resulted in no separation of the anomeric mixture. It was possible in some cases to obtain the pure β-anomer using this technique. The α-anomer appeared to decompose more rapidly than the β-anomer, hence if the elution time was gauged correctly, the β-anomer could be recovered after complete decomposition of the α-anomer had occurred. Not surprisingly the yields of the pure β-anomer obtained using this method never exceeded 10%.

The assignments of the anomeric configurations were based on nuclear Overhauser effect (n.O.e.) experiments on the pure β-anomer (267).



$^3J_{3,4}$ 9.9Hz

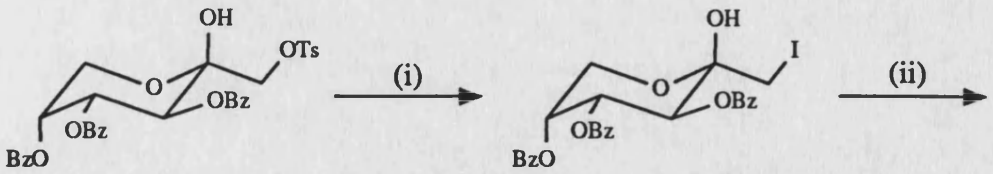
(267)

Figure 16.

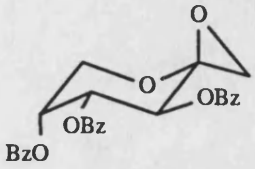
The conformation of the epoxide was deduced from the coupling constant between the 3-H and 4-H protons (9.9Hz), which was consistent with the $^2C_5$ conformation. Irradiation of the methylene protons on C-1 showed an n.O.e. to 3-H, but there was no observable enhancement to the protons on C-4 or C-6 (see **Appendix 1**), indicating the β-configuration (**Figure 16**).

Due to the problems encountered in separating the anomeric mixture of epoxides, an anomerically specific synthesis was required, necessitating ring closure under neutral conditions to prevent tetrahydropyran ring opening.

Displacement of the tosylate leaving group from (268) was readily achieved on treatment with potassium iodide in DMF at 70°C to yield the iodohydrin (270). This is in marked contrast to the earlier experiment with 2,3:4,5-di-O-isopropylidene-1-O-*p*-toluenesulphonyl-β-D-fructopyranose (230), in which no reaction was observed over prolonged reaction time with potassium iodide in DMF at 150°C. This suggests that the benzoate protecting groups lend anchimeric assistance[120] to the displacement of the tosyl group. If the reaction does indeed proceed *via* neighbouring group participation, then benzoate migration to the primary hydroxyl group would be a possibility. However, only a single product was formed and the position of the iodide on C-1 was unequivocal from the observed n.m.r. spectra. The pair of doublets due to the protons on C-1 had shifted upfield from 4.15 and 4.21ppm for the primary tosylate (268) to 3.54 and 3.63ppm



(268) (270)



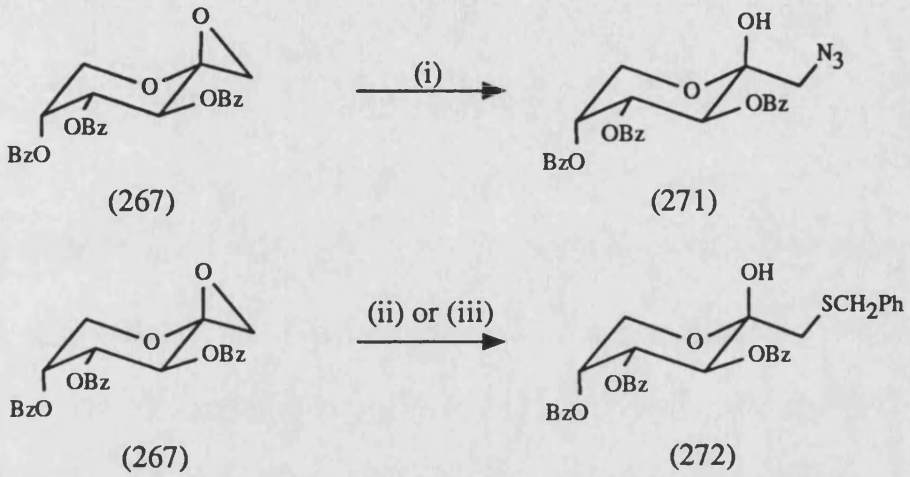
(267)

Scheme 52. *Reagents and conditions:* (i) KI, DMF, 70°, 24h (75%); (ii) Ag_2O, THF, rt, 48h (97%).

for the primary iodide (270). More spectacularly, the C-1 carbon signal had shifted from 70.45 to 14.85ppm, the high field signal being indicative of an iodide. Epoxide formation was then smoothly achieved under neutral conditions on treatment of the iodohydrin (270) with silver (I) oxide to facilitate hydrogen iodide removal[121], affording exclusively the β-anomer (267) (**Scheme 52**). The measured optical rotation of the β-anomer (-273.2°) was considerably more levorotatory than the mixture of α- and β-anomers (-176.7°), which is in agreement with the assignment of the anomeric configuration.

Reactivity of the Anomeric Spiro-epoxide

An investigation of the reactivity of the anomeric spiro-epoxides revealed facile ring-opening with a variety of heteroatom nucleophiles.



Scheme 53. *Reagents and conditions:* (i) NaN_3, DMF, rt, 1h (38%); (ii) NaH, BnSH, DMF, rt, 30min (46%); (iii) BnSH, DBU, PhMe, rt, 1h (55%).

Reaction of the epoxide (267) with sodium azide[122] was complete within 1h at room temperature yielding exclusively the primary azide (271). Similarly reaction of (267) with sodium benzylthiolate[95], or benzylsulphide catalysed with DBU[123], gave the primary sulphide (272), the latter reaction giving a slightly improved yield (**Scheme 53**).

The regioselectivity of ring-opening was determined by comparison of the n.m.r. spectra, the differences being most apparent in the observed ^{13}C spectra (**Table 2**).

The C-1 methylene signal of the azide (271) and, in particular the sulphide (272) occurred at significantly higher field than for the corresponding diol (266) with the alcohol function on C-1. In addition, the signals due to the anomeric carbon were almost identical for all three compounds, and were consistent with an

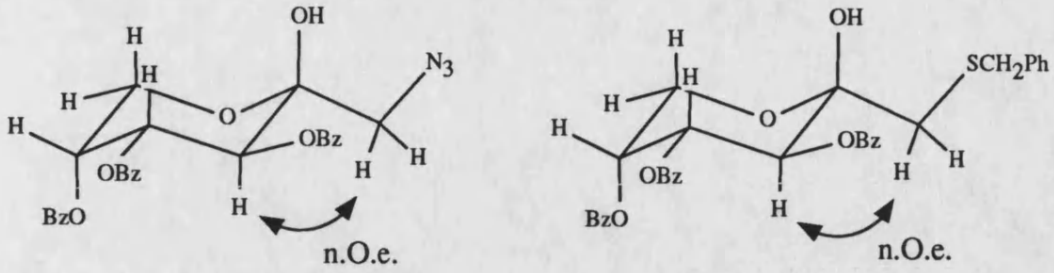
Compound	C-2	C-1 (ppm)
1,2-anhydro-3,4,5-tri-O-benzoyl--β-D-fructopyranose (267)	82.61	50.37
3,4,5-Tri-O-benzoyl -β-D-fructopyranose (266)	97.43	65.49
1-azido-1-deoxy-3,4,5-tri-O-benzoyl-β-D-fructopyranose (271)	97.40	56.05
1-deoxy-thiobenzyl-3,4,5-tri-O-benzoyl-β-D-fructopyranose (272)	97.40	38.40

Table 2

anomeric carbon bearing an hydroxyl group. Thus, as would be expected, base catalysed ring opening had occurred at the least hindered terminal end of the epoxide. The anomeric configurations of (271) and (272) were inferred from their optical rotations, both having similar values to the β-epoxide (267) and the diol (266), implying the β-configuration for both compounds. This was confirmed by n.O.e. experiments.

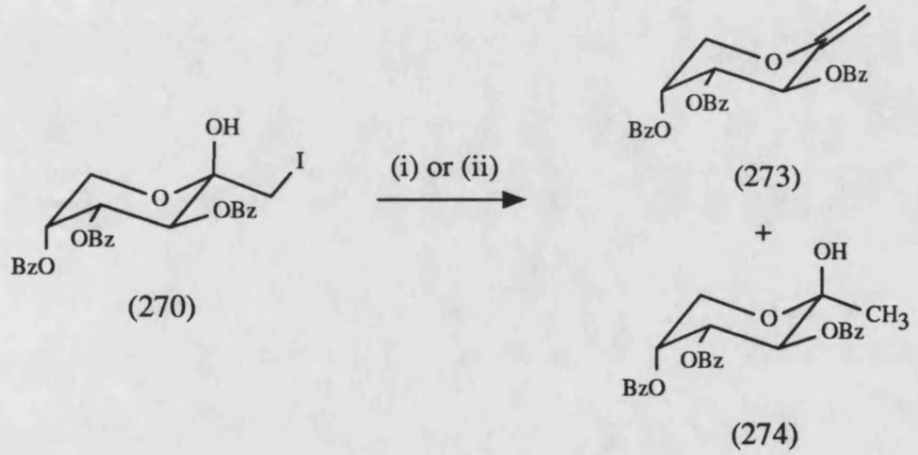
The large coupling constants between the 3-H and 4-H protons for both the azide (271) and the sulphide (272) were consistent with the $^2C_5$ conformation. Irradiation of the methylene protons on C-1 of the azide showed an n.O.e. to 3-H and, in addition, irradiation of the anomeric hydroxyl group showed an n.O.e. to 4-H and 6-H (see **Appendix 2**). Irradiation of the methylene protons on C-1 of the sulphide showed an n.O.e. to 3-H, but there was no observable enhancement to the protons on C-4 and C-6 (see **Appendix 3**) indicating the β-configuration for both compounds (**Figure 17**).

Since the reactivity of 3,4,5-tri-O-benzoyl-1-O-*p*-toluenesulphonyl-



(271) $^3J_{3,4}$ 9.8Hz (272) $^3J_{3,4}$ 10.4Hz

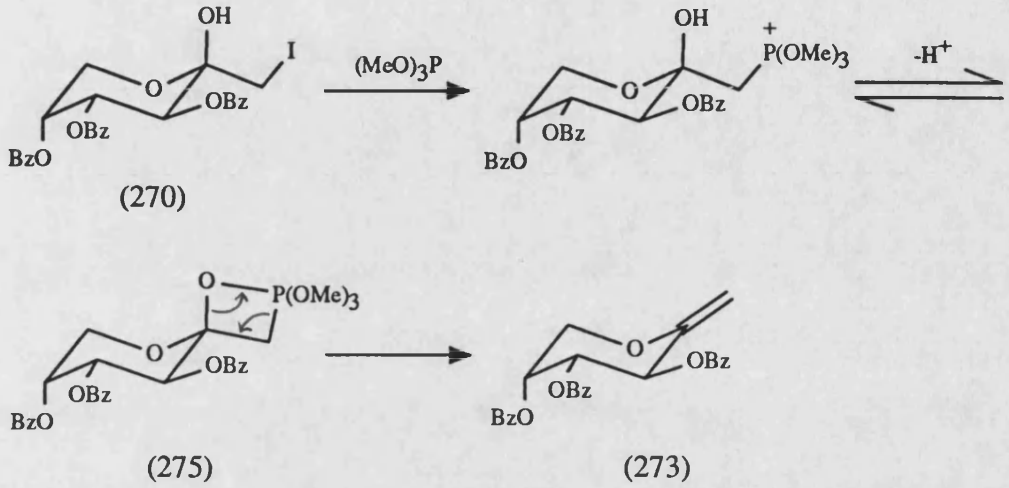
Figure 17.



Scheme 54. *Reagents and conditions:* (i) $(MeO)_3P$, reflux, 12h ((273) 20% and (274) 22%); (ii) NaH, $(EtO)_2P(O)H$, PhMe, reflux, 3h ((273) 10% and (274) 10%).

β-D-fructopyranose (268) was enhanced due to anchimeric assistance, the Michaelis-Arbuzov and Michaelis-Becker reactions were investigated on the corresponding iodohydrin (270) in an attempt to introduce the required phosphonate moiety. The reaction of the iodohydrin in refluxing trimethyl phosphite was complete after 12h, yielding two products, However, neither corresponded to the expected phosphonate. Produced in almost equal amounts

were the vinyl ether (273) and the reduced compound (274) (**Scheme 54**).



Scheme 55.

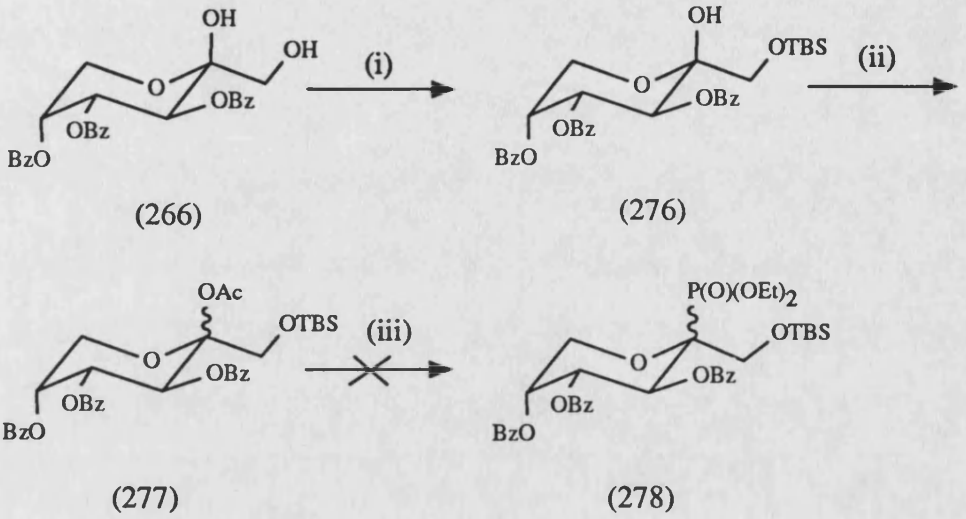
The vinyl ether (273) was characterised by the C-1 methylene protons which resonated as multiplets at 4.70 and 4.90ppm, the C-1 methylene signal had shifted considerably downfield to 154.09ppm and the i.r. spectrum contained a peak corresponding to an alkene at 1664cm^{-1}. The formation of the vinyl ether can best be explained by initial displacement of the iodide by the phosphorus nucleophile. However, instead of following the normal course of the Michaelis-Arbuzov reaction, attack of the β-hydroxyl group on the phosphorus cation could be postulated to form the intermediate oxophosphetane (275). Elimination of trimethyl phosphate, analogous with the elimination of triphenylphosphine oxide in the Wittig reaction[124], would yield the vinyl ether (273) (**Scheme 55**).

The methyl compound (274) was characterised by a singlet in the ^{1}H n.m.r. at 1.59ppm, integrating to three protons, and a methyl resonance in the ^{13}C n.m.r. at 26.17ppm. Compound (274) is presumably formed by reduction of the starting iodohydrin (270).

Similarly, Michaelis-Becker reaction of the iodohydrin (270) with the

sodium salt of diethyl phosphite in refluxing toluene[125] afforded the vinyl ether (273) and the reduced compound (274) both in a 10% yield.

Thus, although it proved possible to displace the iodide leaving group with phosphorus nucleophiles, none of the required phosphonate was obtained due to competing pathways to the Michaelis-Arbuzov mechanism.



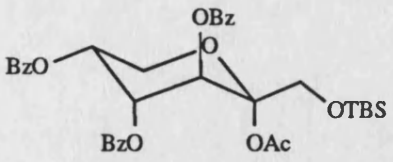
(266) (276)

(277) (278)

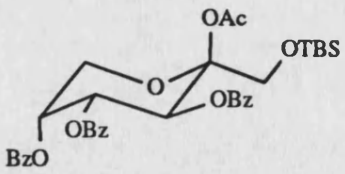
Scheme 56. *Reagents and conditions:* (i) TBSCl, Im, DMF, rt, 18h (86%); (ii) Ac_2O, Py, 80°, 3h (52%); (iii) TMSOTf, $(EtO)_3P$, $CHCl_2$, rt, 24h.

The Meuwly and Vasella[41] methodology for the synthesis of anomeric phosphonates was also investigated. Vasella[76] had exploited this methodology for the synthesis of β-D-fructofuranosyl phosphonate (206), and complementary to this, the synthesis of a D-fructopyranosyl phosphonate (278) was attempted.

The diol (266) was simply mono-protected on the primary hydroxyl group by treatment with *t*-butyl dimethylsilyl chloride and imidazole in DMF[126] at room temperature for 18h. Heating the anomeric alcohol (276) in a 1:1 mixture of acetic anhydride and pyridine at 80°C for 3h gave the 1-O-acetyl-fructopyranose (277) as a mixture of anomers (**Scheme 56**). Interestingly, the acetate (277) was enriched in the α-anomer, the measured α:β ratio being 20:3.



(277α)



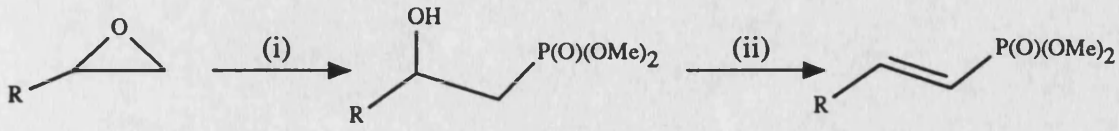
(277β)

The measured optical rotation for the anomeric mixture was +38.5°, indicating that, as expected, the α-anomer was dextrorotatory. The $^5C_2$ conformation for the α-anomer (277α) was indicated by the small 3-H, 4-H coupling constant of 2.0Hz. In this conformation, the α-anomer has the bulky substituent on C-2 equatorial, and the C-O bond axial, both of which are energetically favourable. The β-anomer (277β) had the expected $^2C_5$ conformation as indicated by the large 3-H, 4-H coupling constant of 9.5Hz.

The anomeric acetate (277) however, failed to react under the conditions described by Vasella *et al.*[41, 76] Treatment of the acetate with triethyl phosphite, catalysed with trimethylsilyl trifluoromethanesulphonate in dichloromethane at room temperature yielded only recovered starting material. This is presumably because the oxonium ion was not formed, due to the electron withdrawing benzoate protecting groups. As the reactivity of the spiro-epoxide (267) was now the major focus of the project, the reactivity of the anomeric acetate (277) was not investigated further.

Simple β-hydroxy phosphonates have been synthesised previously by the reaction of epoxides with phosphorus nucleophiles. Baboulene and Sturtz[127] reported the reaction of terminal epoxides (279), where R was a simple alkyl or aryl group, with sodium diethyl phosphite to afford β-hydroxy phosphonates (280) in poor to moderate yields. On prolonged contact with the reagents elimination of water took place to yield the α,β-unsaturated phosphonates (281) (**Scheme 57**).

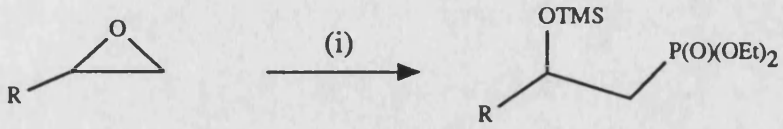
More recently, Okamoto[128] investigated the reaction of terminal alkyl



(279) (280) (281)

Scheme 57. *Reagents and conditions:* (i) $(MeO)_2P(O)Na$, EtOH (15-55%); (ii) Prolonged contact with reagents.

epoxides with diethyl trimethylsilyl phosphite catalysed by Lewis acids. Epoxide ring opening was found to occur exclusively at the terminal C atom to give β-trimethylsiloxy phosphonates (282) in high yields (**Scheme 58**).

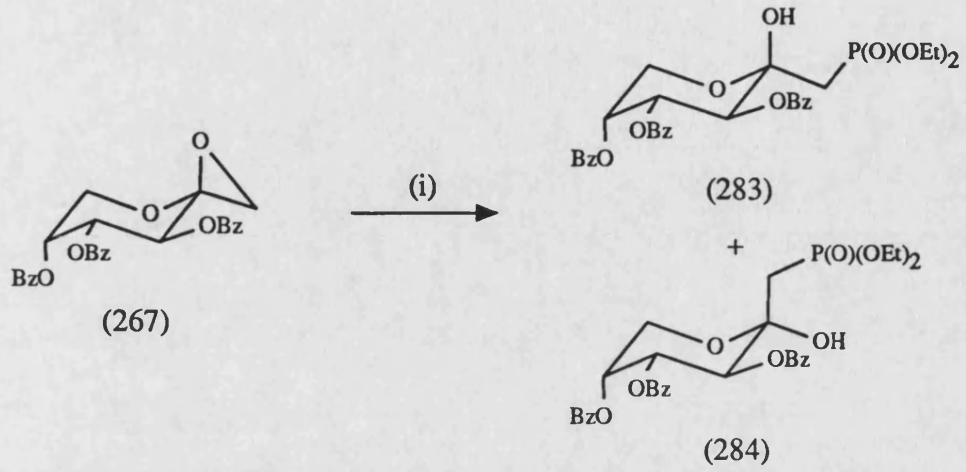


(279) (282)

Scheme 58. *Reagents and conditions:* (i) $(EtO)_2POTMS$, ZnI_2 (1 mol%).

The thermal uncatalysed reactions of trialkyl phosphites with epoxides have also been reported to yield in one case, the corresponding alkene[129], and in another, β-hydroxy phosphonates[130]. The reaction of the spiro-epoxide (267) with triethyl phosphite was initially investigated. Reaction was found to be complete after 2h at 80°C, yielding two products which co-eluted when analysed by t.l.c. in a variety of solvent systems. This proved to be an anomeric mixture of β-hydroxy phosphonates (283) and (284) (**Scheme 59**).

No trace of the vinyl ether (273) was observed, the moderate yield of 55% obtained in the reaction being due to problems encountered in purifying the product from what appeared to be triethyl phosphate, rather than excessive by-product formation. The compounds were characterised by an upfield shift of the



Scheme 59. *Reagents and conditions:* (i) $(EtO)_3P$, 80°, 2h (55%).

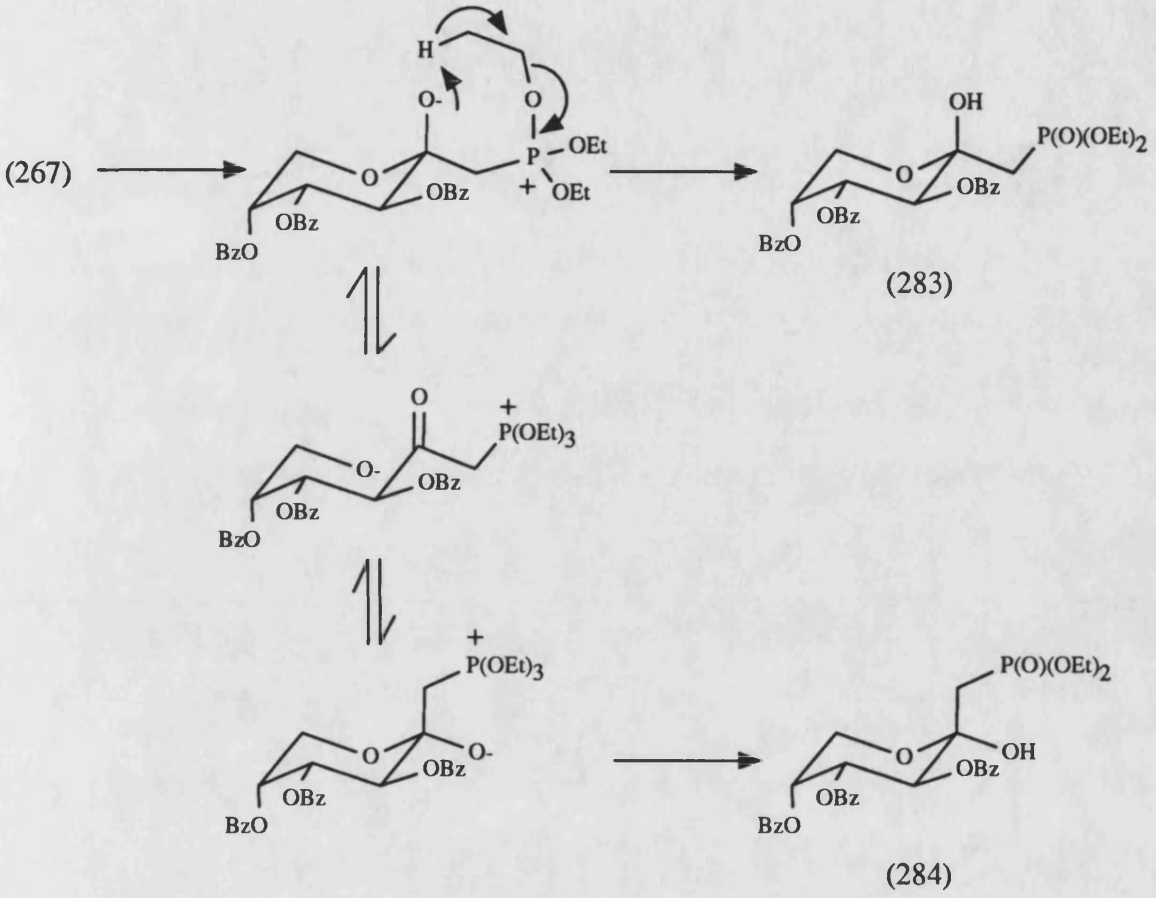
methylene protons on C-1 to give a complex multiplet at 2.29-2.46ppm. The remainder of the proton n.m.r. was complex with overlapping resonances due to the presence of both anomers, however the individual anomers could be identified from the ^{13}C n.m.r. (**Table 3**).

Compound	C-1 ($^1J_{C,P}$)	C-3 ($^3J_{C,P}$)
α-anomer (284)	31.07 (136.6Hz)	82.40ppm (11.0Hz)
β-anomer (283)	33.60 (136.6Hz)	71.83ppm (15.4Hz)

Table 3

The C-1 resonances were also shifted upfield in both anomers, and both showed a coupling to phosphorus of 136.6Hz, consistent with phosphorus bonded directly to the carbon atom. The α:β ratio was 1:2 as measured by n.m.r., and the anomeric configuration assigned by comparison to the pure β-anomer synthesised later. The formation of an anomeric mixture can be explained by the formation of

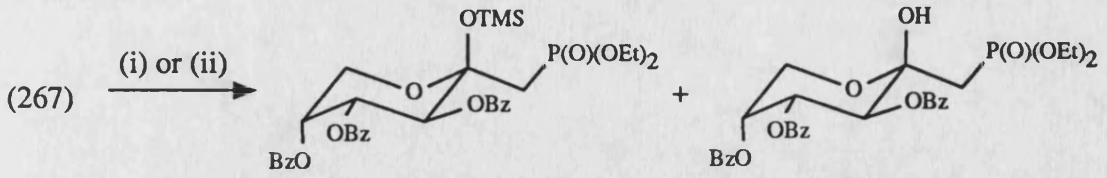
an intermediate betaine. Reversible tetrahydropyran ring opening in competition with ethene elimination would then lead to the formation of a mixture of anomers (**Scheme 60**).



Scheme 60.

Although treatment of the spiro-epoxide (267) with triethyl phosphite had afforded the the required phosphonate, utilising a modification of the Okamoto method[128] proved beneficial.

Reaction of the spiro-epoxide (267) with diethyl trimethylsilyl phosphite catalysed with zinc iodide was complete after 4h at 90° again to yield two products although, in this case, separable by careful flash column chromatography.

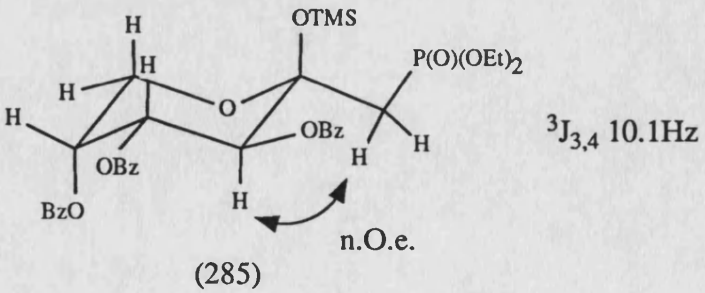


(285) (283)

(i) ZnI_2 catalysed	53%	21%
(ii) Uncatalysed	83%	0%

Scheme 61. *Reagents and conditions:* (i) $(EtO)_2POTMS$, cat. ZnI_2, 90°, 4h; (ii) $(EtO)_2POTMS$, 90°,4h.

The major product was the β-siloxy phosphonate (285) the expected product from the reaction (**Scheme 61**). The C-1 methylene protons had again shifted upfield to give a doublet of doublets at 2.39 and 2.54ppm. The geminal coupling constant was -15.2Hz and, in addition, both resonances showed coupling to phosphorus of 20.6 and 18.7Hz respectively. The C-1 resonance occurred at 36.05ppm and had a phosphorus coupling constant of 138.8Hz. The proton decoupled ^{31}P n.m.r. contained solely a singlet at +22.80ppm the value expected for an alkyl phosphonate.



(285)

Figure 18.

The β-configuration was inferred from the large negative value of the

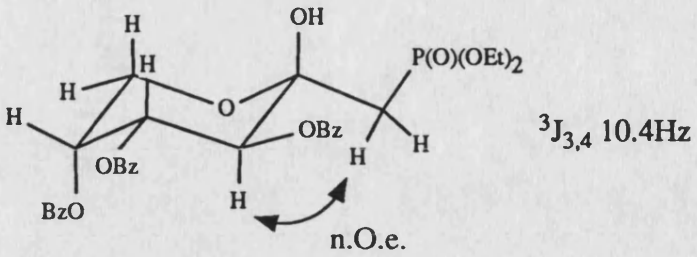
optical rotation, -207.2°, and was confirmed by n.O.e. experiments and subsequently by X-ray crystallography (see **Appendix 4**). The coupling constant between 3-H and 4-H was 10.1Hz implying the diaxial arrangement of the two protons and hence, the expected $^2C_5$ conformation. An n.O.e. enhancement was observed on 3-H on irradiation of the 1-H_2 protons, no change was observed to the 4-H and 6-H protons (see **Appendix 5**), hence, confirming the β-configuration **(Figure 18)**.

The minor product of the reaction isolated in 21% yield was identical to the major product formed in reaction of the spiro-epoxide (267) with triethyl phosphite and was the β-hydroxy phosphonate (283) (**Scheme 61**). The formation of this product can be best explained by Lewis acid catalysed desilylation of the initially formed β-siloxy phosphonate (285). The protons on C-1 were now discernable as a doublet of doublets at 2.31 and 2.39ppm, with a geminal coupling constant of -14.6Hz and with phosphorus coupling of 18.5 and 18.7Hz. The carbon spectrum was identical with that obtained above, and the proton decoupled ^{31}P n.m.r. showed a singlet at +27.55ppm.

Again, the β-configuration was inferred from the large negative value of the optical rotation, -201.3°, which was considerably more levorotatory than the anomeric mixture, -123.4°, obtained earlier, and was confirmed by n.O.e. experiments.

As before, the $^2C_5$ conformation was verified by the large 3-H, 4-H geminal coupling constant and the β-configuration deduced from the n.O.e. between the C-1 methylene protons and 3-H (see **Appendix 6**) **(Figure 19)**.

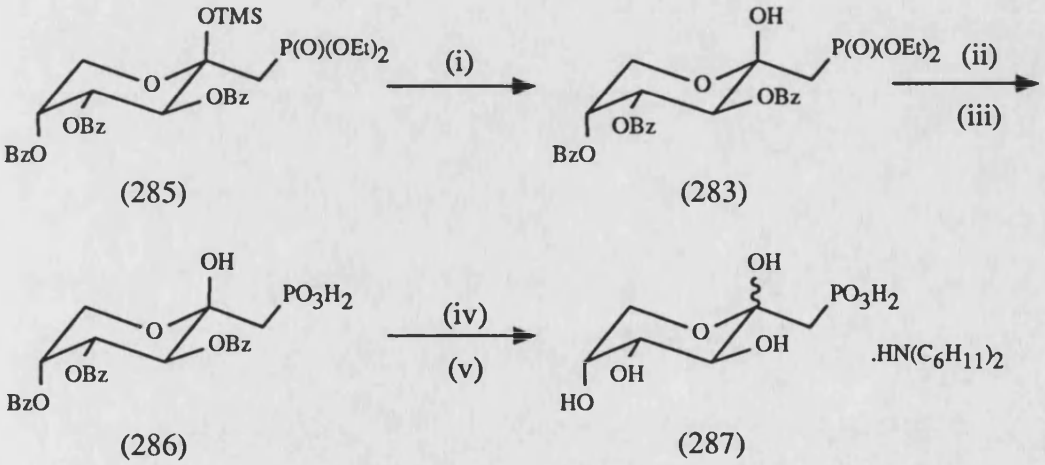
The formation of the β-hydroxy phosphonate (283) by Lewis acid catalysed desilylation of the β-siloxy phosphonate (285) was corroborated by repeating the reaction of the spiro-epoxide (267) with diethyl trimethylsilyl phosphite in the absence of zinc iodide (**Scheme 61**). No reduction in reaction rate was observed, the reaction being complete after 4h at 90°. In this case, the β-siloxy phosphonate



(283)

Figure 19.

(285) was the sole product of the reaction isolated in 83% yield. This method proved the most convenient way to synthesise the primary phosphonate.



(285) (283) (286) (287)

Scheme 62. *Reagents and conditions:* (i) Bu_4NF, THF, 0°, 10min (78%); (ii) TMSBr, CH_2Cl_2, rt, 24h; (iii) H_2O, THF, rt, 2h (98%); (iv) NaOH, MeOH, rt, 2h, Dowex 50(H^+); (v) $(C_6H_{11})_2NH$, MeOH, acetone (22%).

Deprotection of (285) using standard conditions then readily afforded D-fructose 1-deoxy-1-phosphonic acid (287) as its dicyclohexylammonium salt (**Scheme 62**).

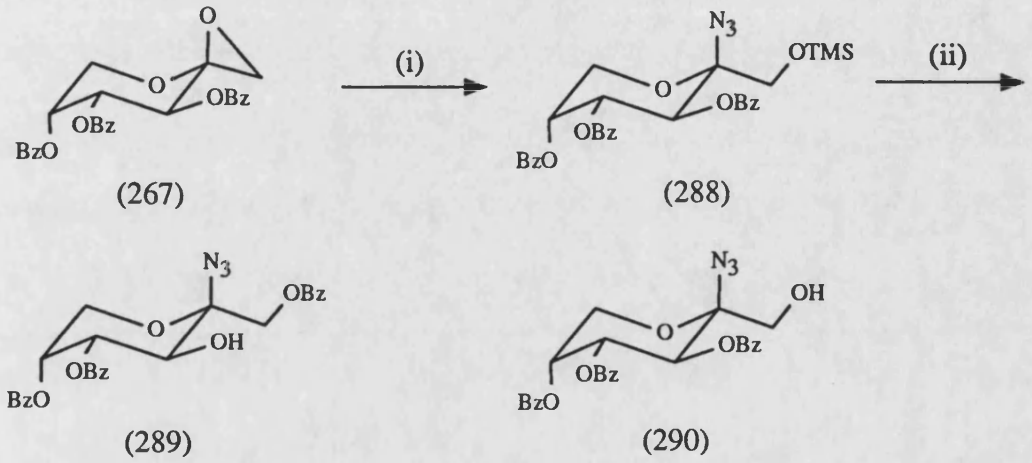
The trimethylsilyl group was readily removed on treatment with 1 equiv. of

tetrabutylammonium fluoride in dry THF[131] at 0°C to afford the β-hydroxy phosphonate (283) solely as the β-anomer. The diethyl phosphonate was transesterified on treatment with 4 equiv. of bromo trimethylsilane[19] in dry dichloromethane over 24h at room temperature. Hydrolysis of the resulting di-trimethylsilyl phosphonate with aqueous THF gave the phosphonic acid (286) as a hygroscopic foam in high yield. The proton n.m.r. clearly lacked the ethyl resonances indicating that complete removal of the ethyl esters had occurred.

The benzoate protecting groups were removed on treatment with 10 equiv. of sodium hydroxide in dry methanol[132] in 2h. Passage of the resulting crude syrup through a column of Dowex 50×8-100 (H^+) ion exchange resin afforded the free phosphonic acid, however, no separation from benzoic acid, the by-product of the reaction, was obtained. Fortunately, it proved possible to remove the benzoic acid by trituration with ethyl acetate. The resulting syrup was dissolved in dry methanol and 0.9 equiv. of dicyclohexylamine added, dilution with acetone precipitated the dicyclohexylammonium salt as a flocculent solid which exhibited a single spot of R_F 0.25 on t.l.c., developed with propanol:ammonia:water 4:3:1. The deprotected phosphonic acid (287) would be present in aqueous solution as an equilibrium mixture of α- and β-pyranose and furanose forms. As such the ^1H and ^{13}C n.m.r. spectra were very complex, but the compound showed the correct molecular ion in F.A.B. mass spectra and gave an adequate elemental analysis (C, 0.6%).

Acid Catalysed Epoxide Ring-opening Reactions

As part of our investigation into the chemistry of anomeric spiro-epoxides we turned our attention to acid catalysed ring opening reactions. It was hoped that this would provide complementary reactivity to the base catalysed ring-openings. As outlined earlier, reaction of the nucleophile at the anomeric centre would represent a strategy for the synthesis of analogues of D-fructose 2-phosphates.



Scheme 63. *Reagents and conditions:* (i) $TMSN_3$, $ZnCl_2$, CH_2Cl_2, rt, 3h (61%); (ii) Bu_4NF, THF, 0°, 10min (61%).

The reaction of the spiro-epoxide (267) with azido trimethylsilane was first investigated. No reaction was evident in the uncatalysed reaction in refluxing dichloromethane after 12h. However, the zinc chloride catalysed reaction[133] was complete in 3h at room temperature, yielding one major and several unidentified minor products. The presence of a siloxy group in the major product was evidenced by a singlet in the ^1H n.m.r., integrating to nine protons, at 0.08ppm. In addition, the i.r. spectrum contained a strong azide stretch at 2110cm^{-1}. The C-1 methylene protons occurred as a broad singlet at 3.95ppm, *ca.* 0.5ppm lower field than for the corresponding primary azide (271), the product of base catalysed

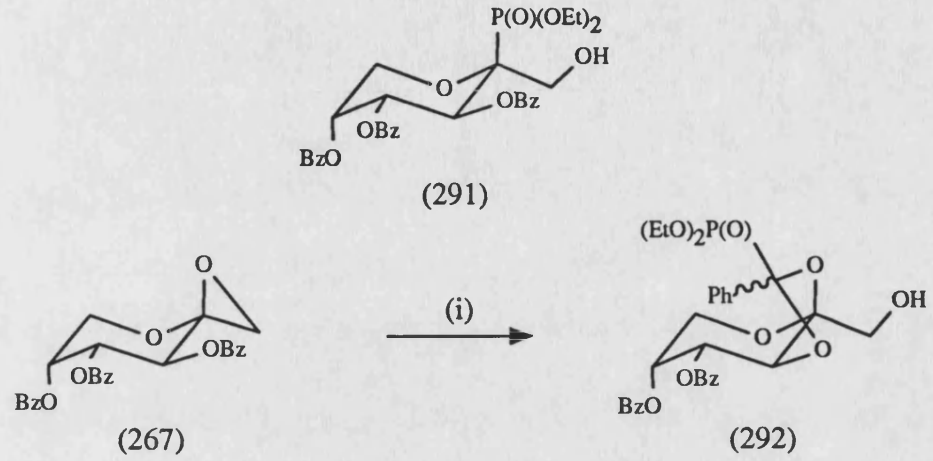
ring-opening. This indicated that, as postulated, epoxide ring-opening had occurred at the more substituted centre to afford the anomeric azide (288) (**Scheme 63**). No n.O.e. data is available for this molecule, but the large negative value for the optical rotation (-239.6°) and the fact that the molecule adopts the $^2C_5$ conformation, as evidenced by the large 3-H, 4-H coupling constant (10.1Hz), both imply the β-configuration.

Complete desilylation was achieved on treatment with tetrabutylammonium fluoride[131] using standard conditions, but led to an unexpected product, the primary benzoate (289). The C-1 methylene protons occurred at 4.78 and 4.88ppm, at much lower field than would be expected for a primary alcohol. In addition, the 3-H resonance had shifted 1.70ppm upfield to 4.40ppm and occurred as a broad triplet, which reduced to a doublet on D_2O shake. It is obvious from the n.m.r. data that bonded to C-3 is now a hydroxyl group, and that the benzoate protecting group has migrated to the less sterically congested primary alcohol. None of the expected primary alcohol (290) was observed. Benzoate migrations of this type have been well documented[134], and partial benzoate migration catalysed by removal of a silyl group with tetrabutylammonium fluoride has been reported[135].

This benzoate migration nicely confirms the position of the trimethylsiloxy group on the primary carbon atom of the starting material (288). The β-configuration of (289) was confirmed by a n.O.e. enhancement of the 3-H proton on irradiation of the C-1 methylene protons (see **Appendix 7**). This also confirms that the initial product, (288), from the Lewis acid catalysed epoxide ring-opening had the β-configuration.

The zinc chloride catalysed reaction of the spiro-epoxide (267) with diethyl trimethylsilyl phosphite as the nucleophile was next investigated. The reaction, complete after 3h at room temperature, yielded a phosphonate as the major product. Little structure data could be gleaned from the ^1H n.m.r., as most of the resonances occurred within a complex multiplet between 4.02 and 4.22ppm.

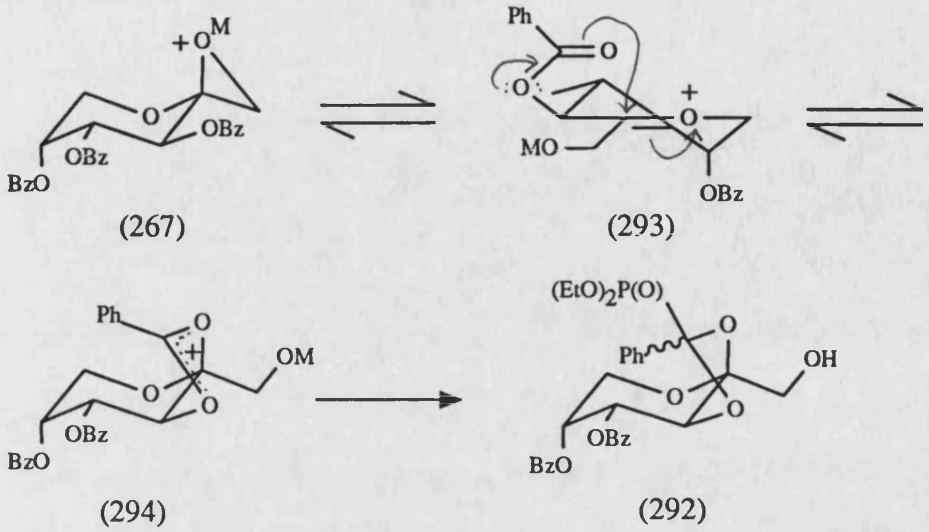
The ^{13}C n.m.r. was clearer, but was not consistent with the expected anomeric phosphonate (291) (**Scheme 64**).



Scheme 64. *Reagents and conditions:* (i) $(EtO)_2POTMS$, $ZnCl_2$, CH_2Cl_2, rt, 3h (47%).

The C-2 resonance for the anomeric phosphonate would be expected as a doublet at *ca.* 85ppm[41, 76]. However, the ^{13}C spectrum contained at low field a singlet at 108.04ppm and a doublet at 109.42ppm with $^1J_{C,P}$ 205.0Hz. The proton decoupled ^{31}P n.m.r. contained a singlet at +12.32ppm, *ca.* 10ppm higher field expected for a simple alkyl phosphonate[136]. Consistent with this data is the 1′-diethyl phosphonate benzylidene (292) (**Scheme 64**). The anomeric carbon occurs as the singlet at 108.04ppm, similar in value to that of the corresponding carbon atom in the di-acetonide (228) (104.47ppm). The doublet at 109.42ppm would then be due to the benzylidene carbon atom and contains the expected large coupling to phosphorus. The up-field shift of the ^{31}P resonance could then be explained by the electron withdrawing effect of the two α-oxygen atoms.

The formation of (292) can be explained by initial formation of the oxonium ion (293) followed by neighbouring group participation of the benzoate group on C-3 to form the resonance stabilised cation (294). Preferential reaction of



(267) (293)

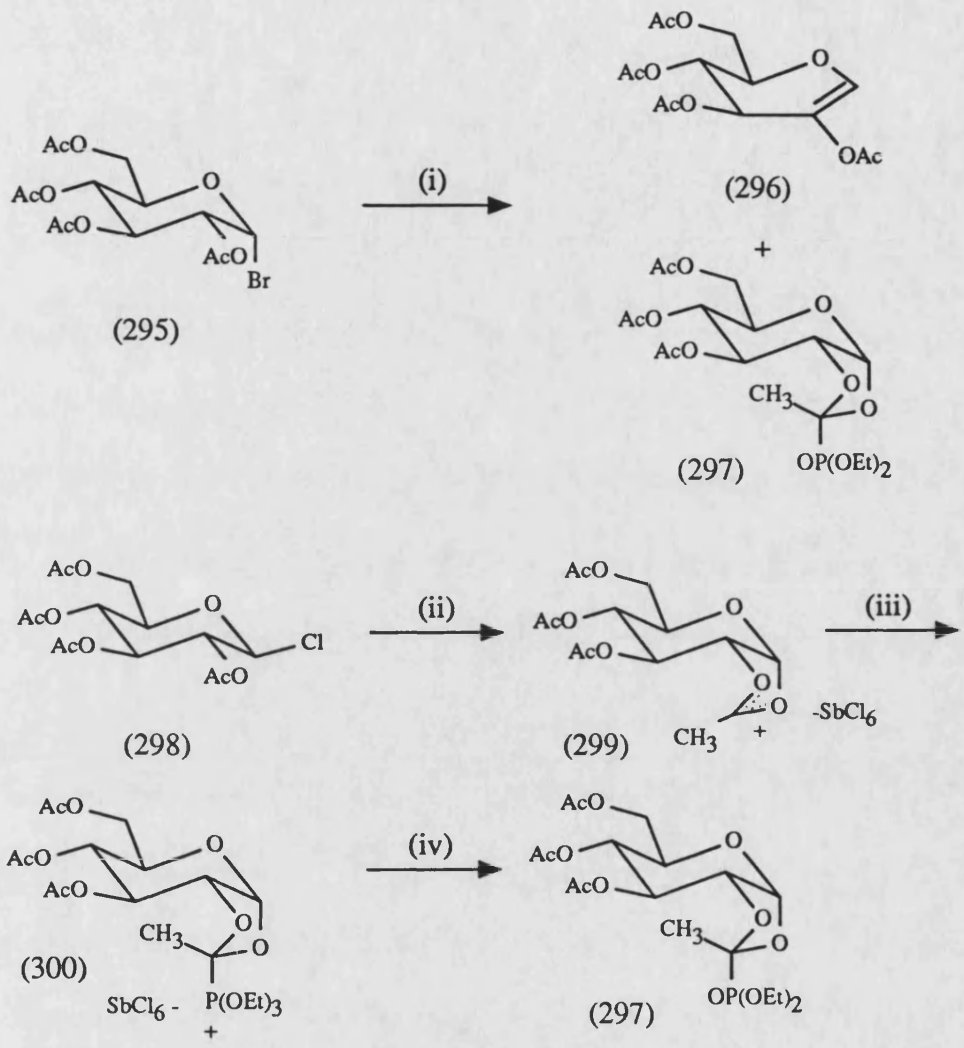
(294) (292)

Scheme 65.

the phosphorus nucleophile at the benzylidene carbon atom would then yield the observed product (**Scheme 65**).

A similar product produced by the participation of a neighbouring ester group has been reported by Paulsen *et al.*[137] Michaelis-Arbuzov reaction of tetra-O-acetyl-α-D-glucopyranosyl bromide (295) with triethyl phosphite yielded the glucal (296) and the phosphonate (297) in a 9:1 ratio (**Scheme 66**).

The phosphonate (297) was also prepared by an alternative strategy[138], treatment of the glucopyranosyl chloride (298) with antimony pentachloride yielded the resonance stabilised cation (299) which reacted with triethyl phosphite to give the phosphonium ion (300). Treatment of (300) with sodium ethoxide then gave the phosphonate (297) (**Scheme 66**).

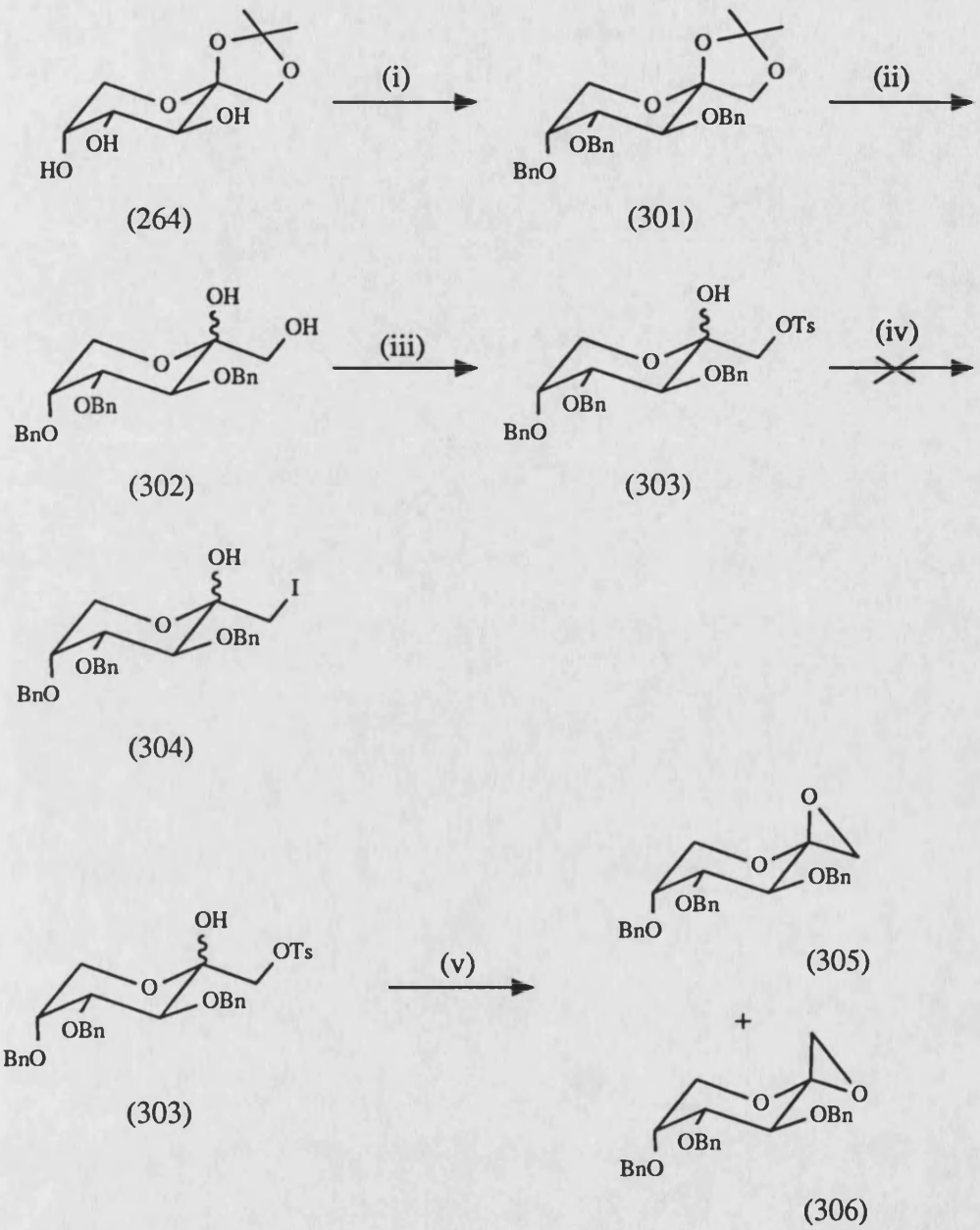


Scheme 66. *Reagents and conditions:* (i) $(EtO)_3P$, reflux (ii) $SbCl_5$ (iii) $(EtO)_3P$ (iv) NaOEt.

Synthesis of Anomeric Spiro-epoxides with 'Non-participating' Protecting Groups

Due to the problems associated with neighbouring group participation of the benzoate protecting groups in Lewis catalysed ring-opening of the spiro-epoxide (267), a synthesis of the spiro-epoxide incorporating non-participating protecting groups was required. The benzyl ether is well known as a non-participating and easily removable protective group in carbohydrate chemistry[139]. However, replacing the benzoate protecting groups with benzyl groups greatly altered the reactivity profile of the carbohydrate moiety. This necessitated a different approach for the synthesis of the benzyl protected spiro-epoxide.

The previously synthesised triol (264) was protected under standard conditions[140] on treatment with 3 equiv. of sodium hydride and benzyl bromide, incorporating tetrabutylammonium iodide as a phase transfer catalyst. As with the corresponding compound incorporating benzoate protecting groups (265), hydrolysis of the remaining acetonide group proved troublesome. A variety of acidic hydrolysis conditions were attempted[141] which failed to cleave the acetonide. As with (265), 50% aqueous trifluoroacetic acid proved to be the reagent of choice. However, yields of the diol (302) were consistently low to moderate and the reaction furnished a mixture of anomers, with an α:β ratio of 1:2.5. The diol reacted with *p*-toluenesulphonyl chloride in pyridine to yield exclusively the primary tosylate (303) with a measured α:β ratio of 1:2.8. Unfortunately, the tosylate (303) now lacking the anchimeric assistance donated by benzoate protecting groups failed to react with potassium iodide at 150°C in DMF over an extended time period. The reaction afforded only starting material and polar decomposition products. The spiro-epoxide could be obtained directly from the tosylate (303). Deprotonation of the anomeric hydroxyl group with potassium



Scheme 67. *Reagents and conditions:* (i) NaH, BnBr, Bu_4NI, THF, rt, 18h (79%); (ii) 50% aq. TFA, rt, 24h (49%); (iii) TsCl, Py, rt, 18h (79%); (iv) KI, DMF, 150°; (v) K^+ *t*-BuO^-, THF, rt, 1h (87%).

tert-butoxide in THF led to intramolecular displacement of the tosylate leaving

group to afford the anomeric epoxides (305) and (306) (**Scheme 67**), with an α:β ratio of 1:6. The change in anomeric ratio can be explained by reversible tetrahydropyran ring opening experienced by the corresponding benzoate protected tosylate (268).

The anomeric spiro-epoxides (305) and (306) proved to be very much more unstable than their counterparts incorporating benzoate protection (267) and (269). The epoxides were especially acid labile, complete decomposition occurring on t.l.c., the formation of the epoxides was characterised by the presence of the corresponding diol (302) on t.l.c. Especial care had to be taken in preparing samples of the spiro-epoxides for n.m.r. analysis. The deuterochloroform had first to be passed through a small column of basic alumina, to remove all traces of hydrochloric acid, before use.

The spiro-epoxides were characterised by a large upfield shift of the C-1 methylene protons in the ^{1}H n.m.r. The α-anomer (306) exhibited an AB system at 2.81ppm, with a small geminal coupling constant of -5.3Hz. The β-anomer (305) exhibited a broad singlet, integrating to two protons, at 2.92ppm. A similar upfield shift of the C-1 and C-2 resonances was observed in the ^{13}C n.m.r. spectrum **(Table 4)**.

Compound	C-2	C-1 (ppm)
1,2-anhydro-3,4,5-tri-O-benzyl--α-D-fructopyranose (306)	82.48	49.46
1,2-anhydro-3,4,5-tri-O-benzyl--β-D-fructopyranose (305)	83.39	50.37

Table 4

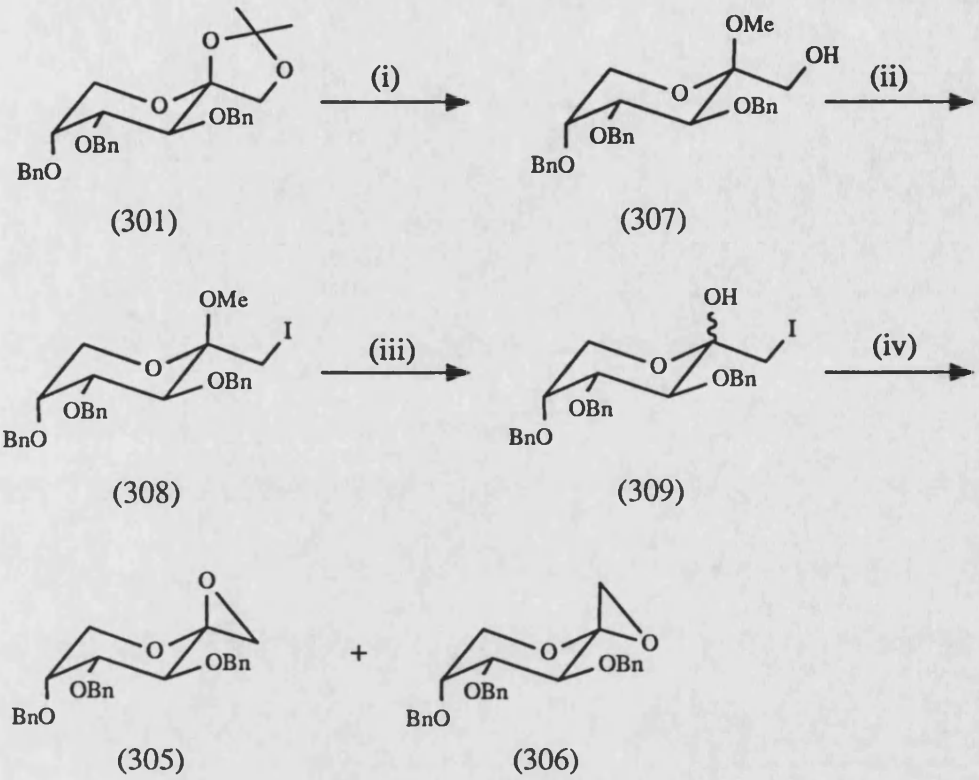
No n.O.e. data is available to assign unequivocally the anomeric configuration. However, the major anomer contained a large 3-H, 4-H coupling

constant of 9.5Hz, which implies a diaxial arrangement of the two protons, and hence the $^2C_5$ conformation. In addition, the value for the optical rotation for the anomeric mixture was negative (-28.3°), both of these observations are consistent with the major anomer having the β-configuration.

Although this route gave access to the spiro epoxides (305) and (306), the first step was often low yielding, and the final epoxidation step gave on all but one occasion, the required epoxides contaminated with varying amounts of the diol (302). It was therefore, desirable to synthesise the epoxides from the corresponding iodohydrin, which on treatment with silver (I) oxide, should yield the epoxides uncontaminated with the diol (302).

As displacement of the primary tosylate with iodide had proved impossible in this system, and attempts to form the primary triflate from the diol (302) had been singularly unsuccessful, yielding a complex mixture of products, a new strategy was required. It had been possible to directly synthesise the primary iodide (232) from the alcohol (228), using iodine and triphenylphosphine in the diacetonide series, where anchimeric assistance is not possible. This appeared to be a possible answer to the current problem. Garegy and Samuelsson's reagent mixture is known to form olefins from vicinal diols[142], so this necessitated protection of the anomeric hydroxyl group.

A facile method for the cleavage of acetals on treatment with a 1% solution of iodine in methanol has been reported[143], which yielded methyl glycosides predominating in the β-anomer on cleavage of anomeric acetals. Indeed, this proved to be the case, the acetonide (301) was refluxed in a 1% w/v iodine solution in dry methanol for 4.5h to yield the methyl fructopyranoside (307) exclusively as the β-anomer in high yield. This not only afforded the required protection of the anomeric hydroxyl group but overcame the problem of the previous low yielding acetonide hydrolysis. The effectiveness of iodine in methanol for this particular acetal cleavage can be gauged by the fact that deprotection with a 1% w/v



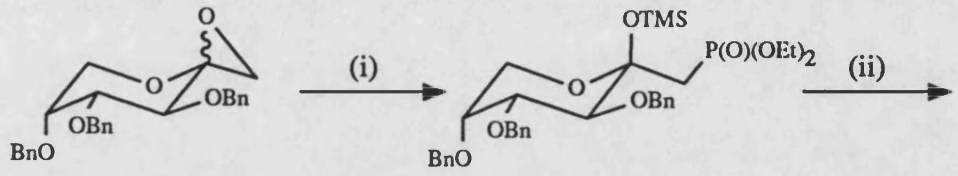
Scheme 68. *Reagents and conditions:* (i) 1% w/v I_2, MeOH, reflux, 4.5h (95%); (ii) I_2, Ph_3P, Im, PhMe, reflux, 5h (69%); (iii) AcOH, H_2O, 100°, 2h (81%); (iv) Ag_2O, THF, rt, 72h (84%).

p-toluene sulphonic acid solution in dry methanol[144] was complete only after 24h at reflux yielding only a moderate 40% yield of the methyl glycoside (307).

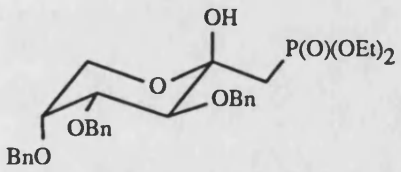
Reaction of the primary alcohol (307) with iodine, triphenylphosphine, and imidazole cleanly afforded the primary iodide (308). The formation of the iodide was characterised by an upfield shift of the C-1 methylene protons and, most dramatically, the C-1 resonance, which occurred at 4.83ppm in the ^{13}C spectrum. The methyl glycoside (308) was hydrolysed under mild conditions on treatment with 80% aqueous acetic acid at 100° to yield the iodohydrin (309), with an α,β ratio of 1:4.5. Epoxide formation was then achieved utilising silver (I) oxide[121] to

give the anomeric spiro-epoxides (305) and (306) (**Scheme 68**), identical with those obtained earlier, with an α:β ratio of 1:5. This route yielded the spiro-epoxides in an overall yield of 45% from the acetonide (301), a significant improvement on the 30% overall yield obtained for the previous synthesis (**Scheme 67**). In addition, the spiro-epoxides derived from the iodohydrin (309) were obtained free from contamination by the diol (302).

Unfortunately, lack of time precluded a thorough investigation of the reactivity of the spiro-epoxides (305) and (306). Facile acid catalysed ring-opening would be expected in the light of the extreme acid lability of these spiro-epoxides.



(305) and (306) (310)



(311)

Scheme 69. *Reagents and conditions:* (i) $(EtO)_2POTMS$, 100°, 5h (50%); (ii) Bu_4NF, THF, 0°, 10min (61%).

The reaction of the spiro-epoxides (305) and (306) with one phosphorus nucleophile was investigated. Thus, treatment of the anomeric mixture with diethyl trimethylsilyl phosphite at 100°C for 3h afforded, after flash column chromatography, the primary phosphonate (310) as a single anomer with β-configuration.

The moderate yield can be explained by the presumed loss of the minor α-anomer and partial desilylation of (310) on chromatography. The C-1 methylene protons of the primary phosphonate (310) occurred as a pair of doublet of doublets at 2.22 and 2.58ppm, with a geminal coupling constant of -15.0Hz, and with proton phosphorus geminal coupling constants of 20.3 and 20.0Hz respectively. The C-1 resonance similarly occurred at high field at 36.07ppm as a doublet of triplets with a phosphorus coupling of 136.6Hz.

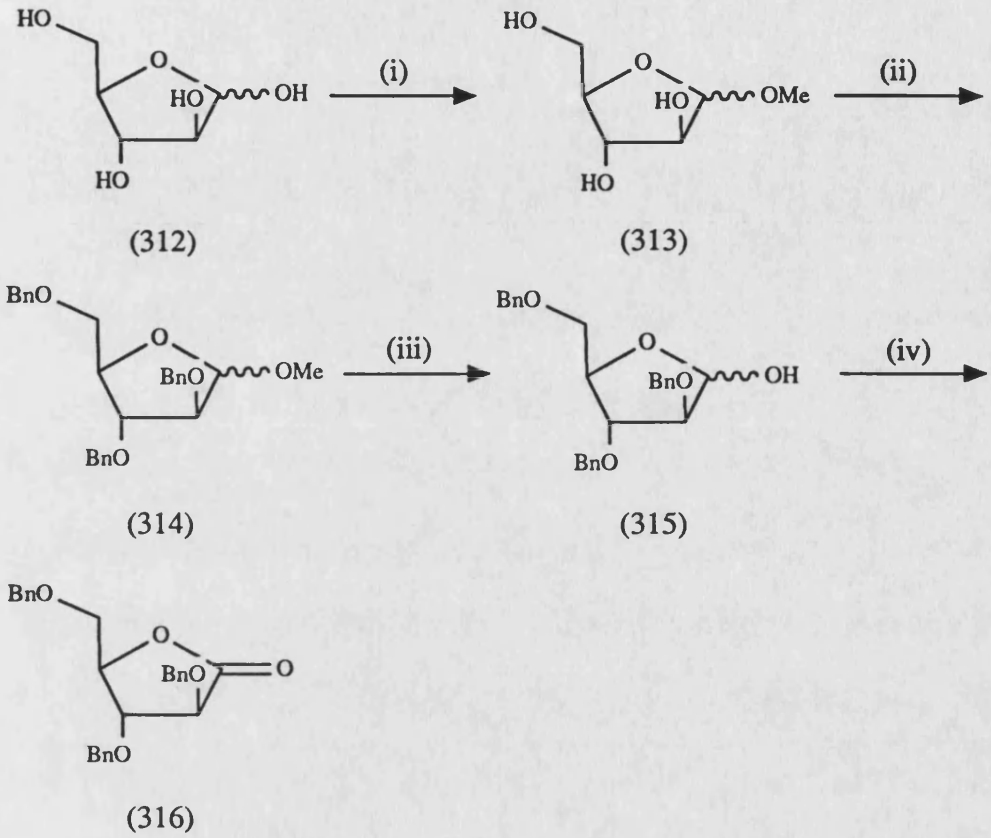
The trimethylsilyl ether (310) was found to be unstable, but could be further characterised after desilylation. Thus, treatment of (310) with 1 equiv. of tetrabutylammonium fluoride in THF at 0°C for 10min cleanly afforded the β-hydroxy phosphonate (311). The loss of the silyl ether and formation of the anomeric alcohol were evident from the ^{1}H n.m.r. and i.r. respectively. A small change was observed in the C-1 methylene protons, which now appeared at 1.83 and 2.39ppm, with a geminal coupling of -15.1Hz and a proton-phosphorus coupling of 18.6 and 17.4Hz respectively. The ^{13}C n.m.r. was little changed, the resonance due to C-1 occurring at 33.28ppm with the phosphorus-carbon coupling constant unchanged at 136.6Hz.

Studies Directed Towards the Synthesis of a Furanose Anomeric Spiro-epoxide

The methodology for the synthesis of pyranose anomeric spiro-epoxides was now well established. Base catalysed ring-opening with nucleophiles at the terminal end of the epoxide can lead to, after deprotection and equilibration, analogues of fructose 1-phosphate (166). However, if epoxide ring opening occurs at the anomeric centre, equilibration is no longer possible, and the carbohydrate is 'trapped' as the pyranose ring. Since fructose 2,6-bisphosphate (167) naturally occurs as a furanose ring, the synthesis of a furanose anomeric spiro-epoxide (246) was highly desirable.

Initial studies towards the synthesis of a furanose epoxide were directed to D-arabinose (312), which exists preferentially as a furanose ring.

The starting point for the synthesis was the readily available 2,3,5-tri-O-benzyl- α/β-D-arabinofuranose[145] (315) synthesised in three steps from D-arabinose in an overall yield of 32%. A solution of D-arabinose in 0.75% v/v sulphuric acid in dry methanol was stirred at room temperature for 15h to afford the methyl glycoside (313). Treatment of (313) with potassium hydroxide and benzyl chloride in refluxing THF yielded the tri-benzyl ether (314). Hydrolysis of (314) with a mixture of 6M hydrochloric acid and glacial acetic acid at 80° gave the anomeric-alcohol (315) of undetermined anomeric configuration (**Scheme 70**). The lactol (315) was found to be readily oxidised with pyridinium chlorochromate in dichloromethane at room temperature to yield the arabinolactone[146] (316). Epoxidation of the lactone (316) would give directly the required furanose anomeric epoxide. Initial attempts were centred on reaction of the lactone (316) with the sulphur ylide dimethyl sulphoxonium methylide[147]. However, the reaction produced largely very polar material, together with a small amount of the α,β-unsaturated lactone (317). The product was characterised by the C=C stretch at

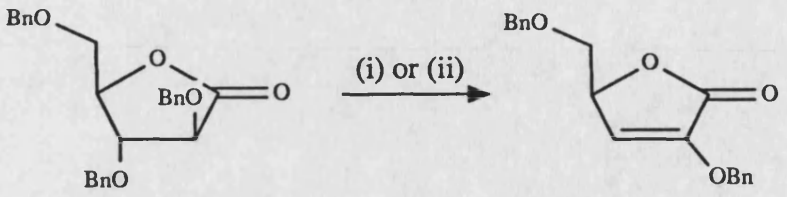


Scheme 70. *Reagents and conditions:* (i) H_2SO_4, MeOH, Drierite, rt, 15h; (ii) KOH, BnCl, Drierite, reflux, 48h; (iii) 6M HCl, AcOH, 80°, 75min (32%); (iv) PCC, CH_2Cl_2, rt, 72h (83%).

1635 cm^{-1} and the C=O stretch at 1761 cm^{-1} indicative of an α,β-unsaturated lactone[148]. It appears deprotonation of the lactone (316) occurs followed by elimination of benzyl alcohol to form the observed product (**Scheme 71**).

Attempts to form the vinyl ether from the lactone (316) by the Wittig reaction[124] with triphenylphosphonium methylide were also unsuccessful, yielding only polar products and a moderate yield of the α,β-unsaturated lactone (317) (**Scheme 71**).

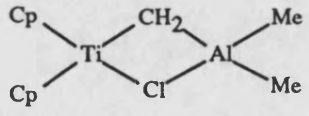
Other strategies remained untried for the epoxidation of (316), either



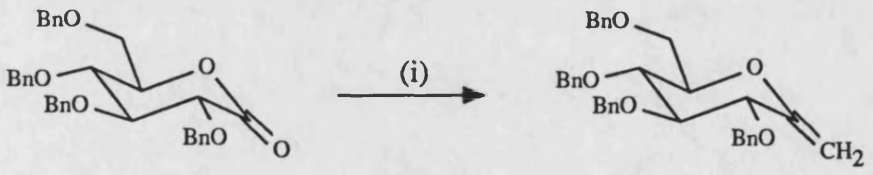
(316) (317)

Scheme 71. *Reagents and conditions:* (i) NaH, $Me_3S{=}O.I^-$, DMSO, rt, 12h (5%); (ii) NaH, $Ph_3PCH_3.I^-$, DMSO, rt, 20h (22%).

directly or *via* the corresponding vinyl ether. Diazomethane has been utilised in carbohydrate chemistry to synthesise spiro-epoxides[149] and, in addition, is known to react with electron deficient esters[150].



(318)



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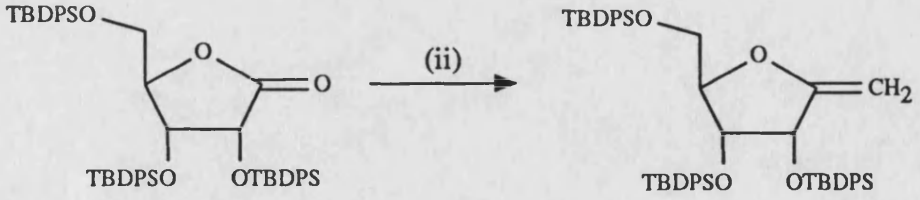


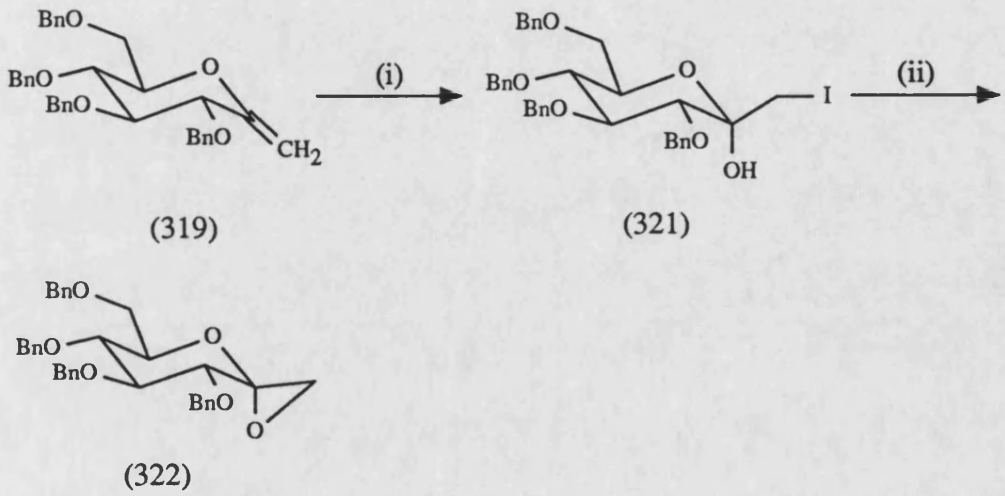
(320)

Scheme 72. *Reagents and conditions:* (i) (318), PhMe, THF, Py, -40° - 0°,1.5h (52%); (ii) (318), PhMe, THF, Py, -40° - 0°, 1h (70%).

Even more optimistically, Rajanbabu and Reddy[151] reported the facile

formation of vinyl ethers from sugar lactones on reaction with Tebbe's reagent[152] (318). Using this method the vinyl ethers derived from D-glucose (319) and D-ribose (320) were obtained in good yield (**Scheme 72**).

However, our attempts to synthesise a furanose spiro-epoxide from the arabinolactone were brought to an abrupt halt on the publication, by van Boom *et al.*[153], of a synthesis of an anomeric spiro-epoxide from the vinyl ether (319) synthesised previously by Rajanbabu[151].

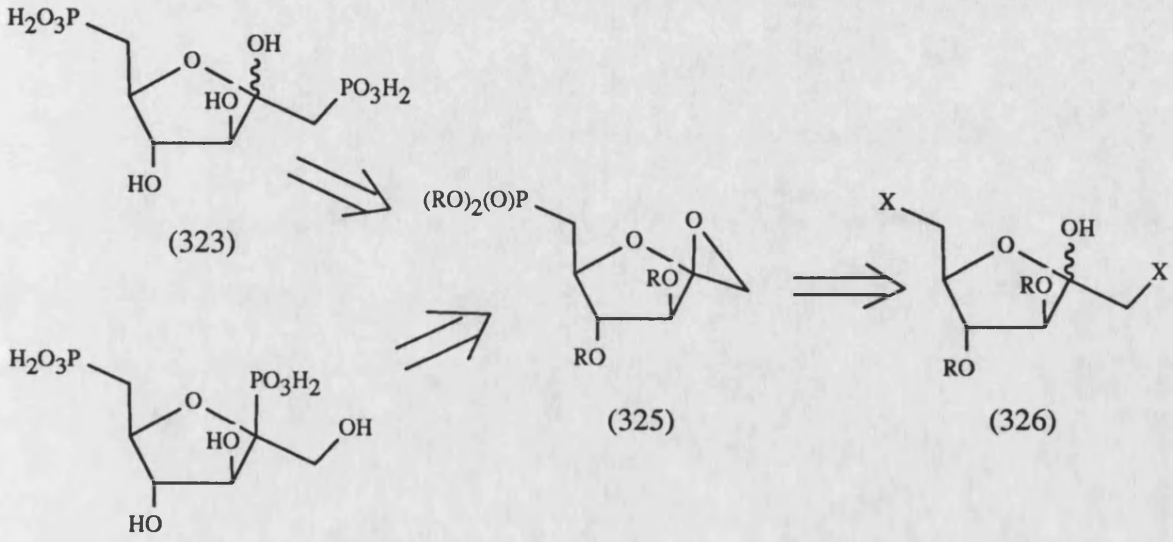


Scheme 73. *Reagents and conditions:* (i) IDCP, H_2O, Et_2O, $ClCH_2CH_2Cl$, rt, 20min (98%); (ii) Amberlite 400 IRA(OH^-), MeOH (96%).

Reaction of the vinyl ether (319) with iodonium *sym*-dicollidine perchlorate (IDCP)[154] in the presence of water afforded the iodohydrin (321) exclusively as the α-anomer. Addition of a slight excess of Amberlite 400 IRA(-OH) ion exchange resin to a solution of (321) in methanol gave the anomerically pure epoxide (322).

Studies Towards the Synthesis of a Furanose Spiro-epoxide from D-Fructose

An alternative strategy for the synthesis of a furanose spiro-epoxide was envisaged from D-fructose. This route contained a common intermediate which could conceivably be transformed directly into bisphosphonate analogues of either fructose 1,6-bisphosphate or fructose 2,6-bisphosphate.

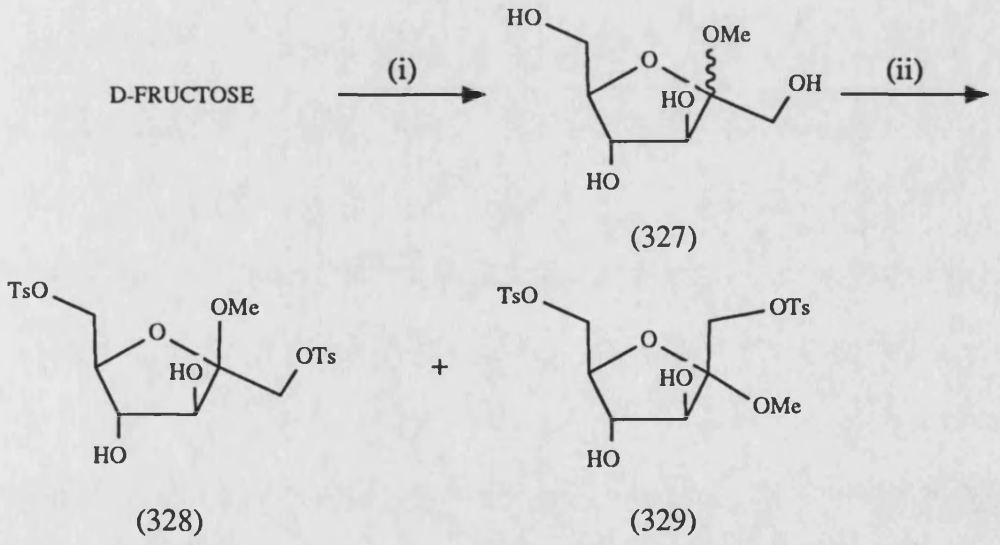


Scheme 74.

This common intermediate was the suitably protected spiro-epoxide (325), containing a 6-phosphonate group. Due to the problems encountered in neighbouring group participation in the pyranose series, the protecting groups of choice were non-participating benzyl ethers. Acid catalysed ring-opening, with a suitable phosphorus nucleophile, would then lead to a bisphosphonate analogue (324) of fructose 2,6-bisphosphate. Alternatively, base catalysed ring-opening would lead to a bisphosphonate analogue (323) of fructose 1,6-bisphosphate. The spiro-epoxide could be synthesised from a substrate such as (326), in which X is a suitable leaving group. It should be possible to exploit the inherent increased

reactivity of a leaving group on C-6, compared to C-1, to selectively incorporate the first phosphonate group on C-6. The synthesis of (326) requires the differentiation of the two primary hydroxyl groups from the two secondary hydroxyl groups on the furanose ring.

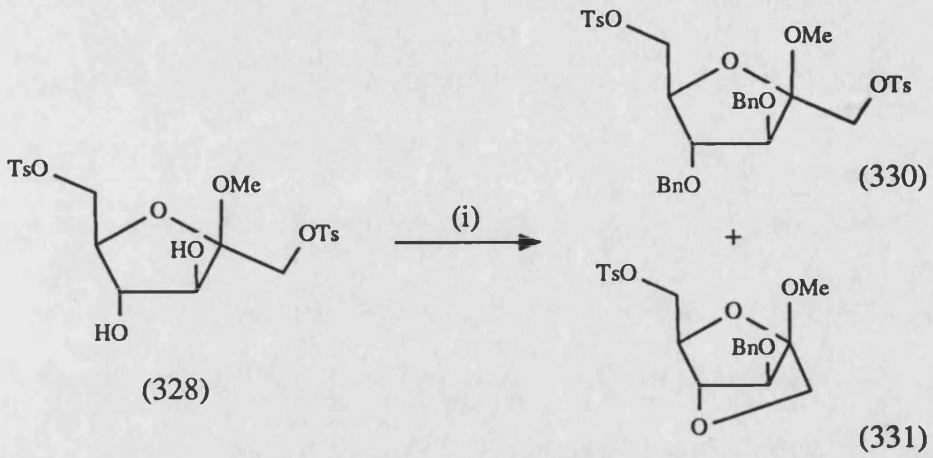
Fischer glycosidation of D-fructose in acidic methanol leads to the formation of an anomeric mixture of methyl fructofuranosides[155](327). Initially, the selective protection of the two primary hydroxyl groups of the methyl furanosides as either trityl[156] or TBDMS[157] ethers was attempted. Both of these groups selectively protect primary hydroxyl groups in the presence of secondary hydroxyl groups[158]. However, none of the required di-protected product could be isolated in either case.



Scheme 75. *Reagents and conditions:* (i) 0.4% v/v H_2SO_4, MeOH, rt, 30min, (ii) TsCl, Py, 4°, 108h (α-anomer 8%, β-anomer 20%).

The primary hydroxyl groups had been shown to react preferentially with *p*-toluenesulphonyl chloride by Wright[159], this had the advantage of directly transforming the 1- and 6-hydroxyl groups into leaving groups. Thus, reaction of

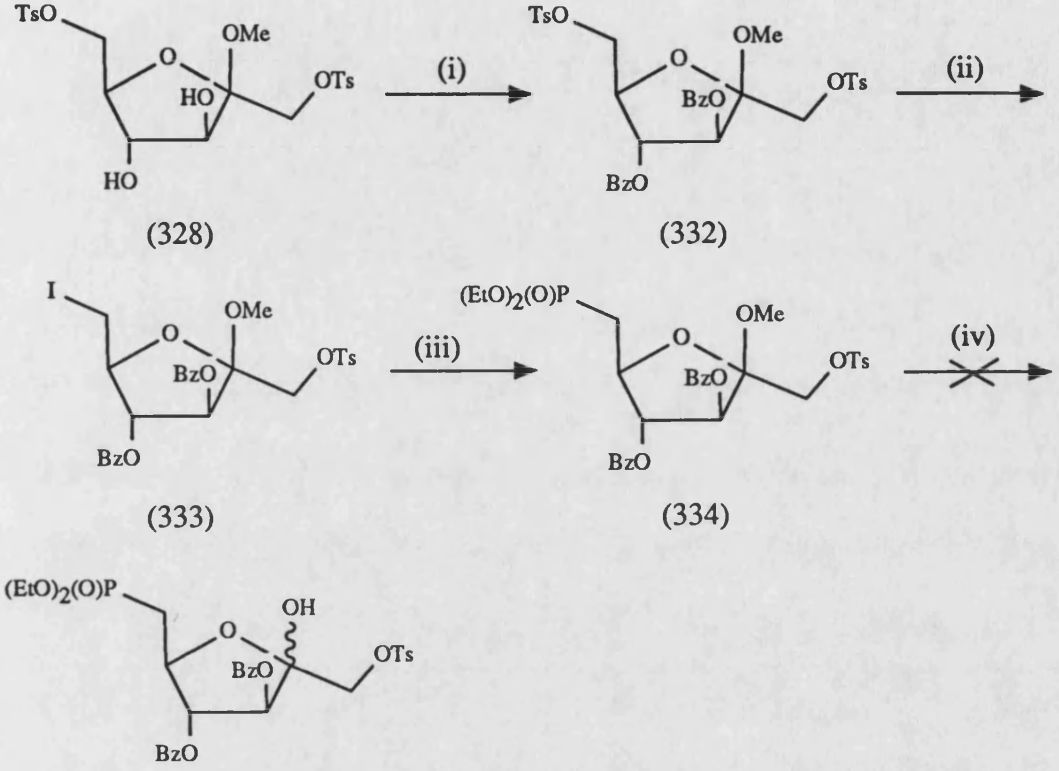
the methyl fructofuranoside (327) with two equiv. of *p*-toluenesulphonyl chloride in pyridine for 108h at 4°C yielded the separable α- and β-1,6-di-tosylates (329) and (328) in 8 and 20% yield respectively (**Scheme 75**). The β-anomer was then carried through the rest of the synthesis.



Scheme 76. *Reagents and conditions:* (i) NaH, BnBr, Bu_4NI, THF, rt, 48h ((330) 14%, (331) 20%).

Protection of the diol (328) as the di-benzyl ether (330) did not prove as simple as expected. Reaction of (328) with sodium hydride and benzyl bromide under standard conditions gave only a poor yield of the di-benzyl ether (330), the major product was an anhydro-sugar (331). The fact that (331) contained only one tosyl and one benzyl group was obvious from the ^1H n.m.r., and the i.r. spectrum did not contain an OH stretch. Which tosyl group had been displaced was not obvious from chemical shift differences, but was determined by the small geminal coupling constant of the C-1 methylene protons of -2.2Hz, indicating (331) was the 1,4-anhydro sugar. Reaction of the methyl furanoside (328) with benzyl bromide and silver (I) oxide[160] in THF at room temperature gave after an extended reaction time, a complex mixture of reaction products, of which the required di-benzyl ether

(330) was only a minor component.



Scheme 77. *Reagents and conditions:* (i) BzCl, Py, 80°, 2.5h (73%); (ii) KI, DMF, 110°, 36h (79%); (iii) $(EtO)_2POTMS$, 140°, 48h (21%); (iv) H^+/H_2O.

In contrast, the di-benzoate ester was readily formed under standard conditions. Reaction of the diol (328) with two equiv. of benzoyl chloride in pyridine at 80° cleanly gave the di-benzoate (332). The 6-tosylate group was known to be easily displaced by nucleophiles[159], whereas the 1-tosylate is of the neopentyl type, and is much less readily displaced. Reaction of the di-tosylate (332) with potassium iodide at 110°C in DMF gave the 6-deoxy 6-iodo compound (333), no displacement of the 1-tosylate group was observed. The iodide (333) was characterised by the high field methylene carbon at 5.58ppm in the ^{13}C n.m.r.,

corresponding to C-6. The iodide though proved unexpectedly resistant to Michaelis-Arbuzov reaction. Treatment with refluxing triethyl phosphite over an extended time period yielded only the starting material and non-phosphorus containing decomposition products. The required 6-phosphonate could be prepared in a low but reproducible 20% yield, by reaction of the iodide (333) with the more nucleophilic diethyl trimethylsilyl phosphite[161] (**Scheme 77**). The C-6 carbon of the C-6 phosphonate (334) appeared as a doublet of triplets with a coupling to phosphorus of 141.0Hz at 31.92ppm in the ^{13}C n.m.r. The C-5 carbon atom was also coupled to phosphorus with a coupling constant of 15.4Hz.

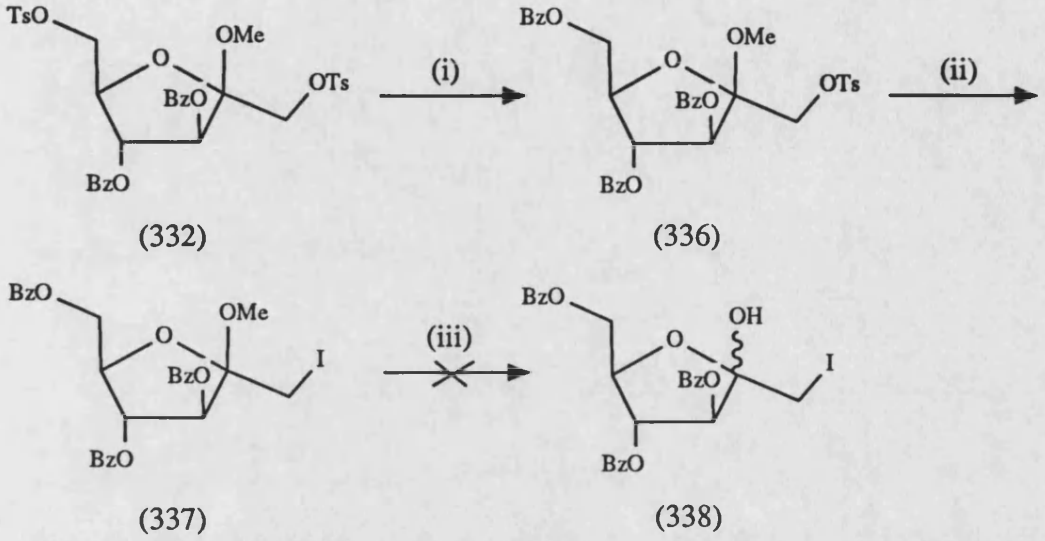
Conditions	(334)	(337)
50% aq. TFA, reflux	no reaction	-
90% aq. AcOH, reflux	no reaction	no reaction
1M H_2SO_4, reflux	no reaction	-
1M H_2SO_4/THF, reflux	no reaction	-
3M H_2SO_4/THF, reflux	no reaction	no reaction
5M H_2SO_4/THF, reflux	decomposition	-
TMSI, CH_2Cl_2, rt	-	no reaction

Table 5

Hydrolysis of the methyl glycoside to yield the β-hydroxy tosylate (335) followed by base catalysed intramolecular displacement of the tosyl group should yield the required anomeric spiro-epoxide. Unfortunately, the methyl glycoside proved resistant to all hydrolysis attempts (**Table 5**).

No reaction was observed under increasingly drastic acidic conditions, finally refluxing in a 1:1 mixture of 5M sulphuric acid:THF for 18h, afforded *ca.* 20% reaction. However, the ^1H n.m.r. of the crude reaction mixture indicated that

the developing product still contained a methoxy group!



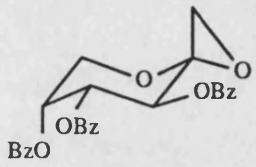
Scheme 78. *Reagents and conditions:* (i) NaOBz, DMF, 90°, 16h (99%); (ii) KI, DMF, 150°, 7days (49%); (iii) H^+/H_2O.

As with the unreactivity of the anomeric acetate (280), this indicates a reluctance to form an oxonium ion. The formation of an oxonium ion is well known to be disfavoured due to the inductive effect of electron withdrawing groups situated around the ring[162]. The presence of polar groups at C-3 (C-2 of aldoses) would be expected to have a particularly large effect on the rate of hydrolysis[163]. Investigation as to whether hydrolysis of the methyl glycoside could be achieved on replacement of the 1-tosylate group, with a less electron withdrawing group, was performed on a model system. The 6-tosyl group of (332) was readily displaced with sodium benzoate in DMF at 90°C to give the tri-benzoate (336). The more sterically hindered 1-tosyl group was found to be displaced on reaction with potassium iodide in DMF at 150°C for 7days, to provide the primary iodide (337) in moderate yield (**Scheme 78**). Compound (337) proved as resistant to acid hydrolysis as (334), (**Table 5**). Demethylation of (337) was not

achieved on treatment with trimethylsilyl iodide in dichloromethane at room temperature either, a reaction that does not proceed *via* the oxonium ion[164]. It is obvious from these results that the synthesis of the furanose anomeric spiro-epoxide is not viable from the methyl fructofuranosides. One possible answer to this problem would be to repeat the synthesis starting with a benzyl fructofuranoside[165]. The benzyl acetal could then be cleaved on hydrogenation, but lack of time precluded investigation of this option.

Summary

The synthesis of spiro-anomeric epoxides, a previously unreported class of compounds, has been developed. Depending on the conditions used for epoxide formation, the fructopyranose epoxides can be obtained as a mixture of α-(269) and β-(267) anomers, or exclusively as the β-anomer.



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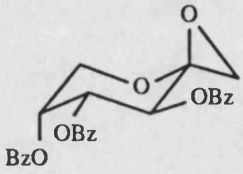
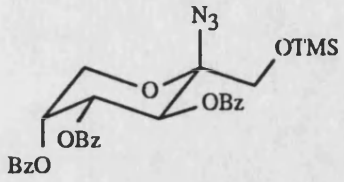


(267)

It has also been shown that reaction of these carbohydrate epoxides with phosphorus nucleophiles allowed access to phosphonates unobtainable from Michaelis-Arbuzov or Michaelis-Becker methodology. Conversion of the resultant β-siloxy phosphonate to an isopolar phosphonate analogue of D-fructose 1-phosphate was then readily achieved. This is, to our knowledge, the first utilisation of carbohydrate epoxides as precursors of phosphonates.

In addition, the acid and base catalysed ring opening of the spiro-anomeric epoxide (267) was investigated with a variety of heteroatom nucleophiles. The base catalysed reaction was found to occur exclusively at the least hindered carbon atom, and in every case but one, afforded solely the β-anomer, as evidenced by n.O.e. experiments.

The outcome of the Lewis acid catalysed reactions were dependant on the nucleophile used. The reaction with trimethylsilyl azide gave an anomeric azide (288). However, the reaction with diethyl trimethylsilyl phosphite gave a 1′-diethyl phosphonate benzylidene (292), as a single diastereomer, due to neighbouring group participation.



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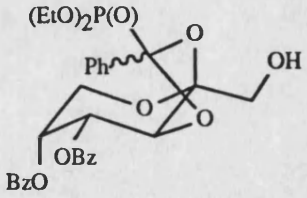
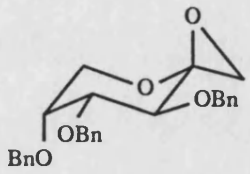


(292)

The route to the spiro-anomeric epoxides with 'non-participating' benzyl protecting groups, (305) and (306), was also developed.



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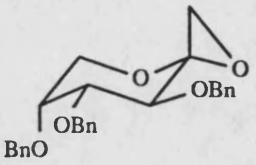
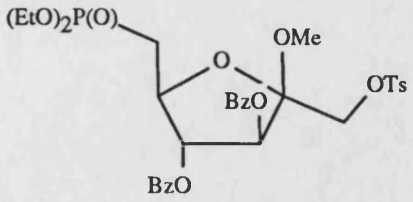


(306)

A comparative study of the acid catalysed reaction of the epoxides (267) and (305) with nucleophiles would be an interesting study.



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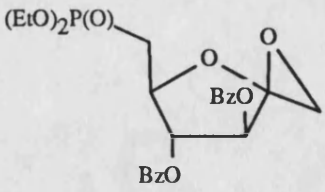


(339)

Time did not permit the synthesis of a furanose anomeric epoxide. However, the first synthesis of a protected isopolar analogue (334) of fructose 6-phosphate was achieved.

Simply changing the anomeric hydroxyl protecting group should allow access to the highly functionalised spiro-anomeric epoxide (339) utilising the

methodology developed. Reaction with phosphorus nucleophiles would then afford bisphosphonate analogues of fructose 1,6-bisphosphate and fructose 2,6-bisphosphate.

EXPERIMENTAL

Instrumentation and Experimental Techniques

Infrared spectra were recorded in the range 4000 - 600 cm^{-1} using a Perkin-Elmer 1310 grating spectrophotometer and peaks are reported (ν_{max}) in wavenumbers (cm^{-1}). Spectra of liquid samples were taken as thin films on sodium chloride plates. Spectra of solid samples were taken as nujol mulls or in chloroform solution, as indicated.

Routine mass spectra from both electron ionisation (EI) and chemical ionisation (CI), were recorded with a VG Analytical 7070E instrument with a VG 2000 data system. Unless otherwise stated the data provided is that from electron ionisation and was produced with an ionising potential of 70eV. Chemical ionisation was conducted with either isobutane or ammonia as the reagent gas. Where possible, the molecular peak (M^+) and base peak are indicated, as are all sizeable fragments with assignments.

Proton magnetic resonance (^1H nmr) spectra were recorded at 270 MHz on a Jeol GNM GX FT 270 spectrometer. Carbon 13 magnetic resonance (^{13}C nmr) spectra were recorded on a Jeol GNM GX FT 270 spectrometer operating at 68 MHz and using 90 and 135 DEPT pulse sequences to aid in multiplicity determination. Proton and ^{13}C nmr spectra were recorded, unless otherwise stated, in $CDCl_3$, and are expressed in parts per million (δ) downfield from internal tetramethylsilane. Multiplicities are given as follows: singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). The abbreviation 'br' is appended to a multiplicity to indicate significant broadening.

Melting points (m.p.) were determined on commercially available apparatus (Gallenkamp) and are uncorrected. Elemental microanalyses were carried out using a Carlo Erba 1106 Elemental Analyser. Optical rotations were measured using a

Perkin-Elmer 141 polarimeter with concentration (c) expressed in g/100 cm^3.

Thin layer chromatography (t.l.c.) was used extensively as a qualitative guide during reactions and for assessing the purity of compounds. Merck DC-alufolien Kieselgel 60 F_{254} sheets containing fluorescent indicator were used for this purpose. Visualisation of reaction components was achieved by illumination under short wavelength (254 nm) ultraviolet light (when possible), and developing with a 7% w/v methanol solution of *dodeca*-molybdophosphoric acid (PMA) followed by warming of the t.l.c. plate.

Medium pressure flash column chromatography was routinely employed using Kieselgel 60 (Merck 9385) and 60H silica gel (Merck 7736) for reaction component separations. A pressure gradient was developed using a small, commercially available hand bellow (Gallenkamp). Material to be chromatographed was preadsorbed onto the column support and applied as a thin layer to the top of the column. Preparative thin layer chromatography was performed using Merck 60 F_{254} silica gel, glass supported plates.

Tetrahydrofuran (THF) was pre-dried over sodium wire, then refluxed over sodium benzophenone ketyl under dry nitrogen until anhydrous. This was redistilled immediately prior to use.

Glassware used for water sensitive reactions was baked in an oven at 120°C for approximately 12h and allowed to cool in a desiccator over $CaCl_2$. Flasks and stirrer bars were, however, additionally flame dried under a stream of dry nitrogen.

In all experiments, the excess solvent was evaporated with a Büchi rotary evaporator using a water aspirator at room temperature to avoid unnecessary heating. All yields quoted are of purified products, and are uncorrected.

All other general reagents and solvents were purified and dried when required, using the methods described in D.D. Perrin, W.L.F. Armarego and D.R. Perrin, 'Purification of Laboratory Chemicals', 2nd Edn., Pergamon Press, Oxford, 1980.

2,3 : 4,5 Di-O-isopropylidene-β-D-fructopyranose (228)

To a solution of conc. sulphuric acid (25 cm^3) in acetone (470 cm^3) at 0°C was added dry, finely powdered D-fructose (25 g, 0.14 mol), and the resulting solution stirred, with exclusion of moisture, at room temperature, for 90 min. The reaction mixture was cooled to 0°C and an ice-cold solution of sodium hydroxide (41.2 g, 1.1 equiv) in water (350 cm^3) was added gradually. The acetone was removed under reduced pressure, and the resulting aqueous suspension extracted with dichloromethane (3 x 300 cm^3). The combined organic extracts were washed with water, dried ($MgSO_4$), and concentrated under reduced pressure to afford a crystalline solid. Recrystallisation from diethyl ether - light petroleum (b.p. 60-80°C) yielded the *protected pyranose* (228) (25.25 g, 70%) as colourless needles, m.p. 97-98°C (lit.,[83] 97°C) (Found : C, 55.5; H, 7.9. calc. for $C_{12}H_{20}O_6$: C, 55.4; H, 7.75%); $[\alpha]_D$ - 23.8° (*c* 1.05 in $CHCl_3$); υ_{max} ($CHCl_3$) 3561 and 3467 (OH), 2957, and 2911 (sat. CH), 1540, 1374, 1241, 1164, 1103, 1065, and 912 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.36 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.55 (3H, s, CH_3), 2.21 (1H, br s, O*H*), 3.65 (1H, d, $J_{1,1'}$ - 11.7 Hz, 1-H), 3.70 (1H, d, $J_{1',1}$ - 11.7 Hz, 1′-H), 3.78 (1H, dd, $J_{6,5}$ 0.9 Hz and $J_{6,6'}$ - 13.0 Hz, 6-H), 3.93 (1H, dd, $J_{6',5}$ 2.0 Hz and $J_{6'6}$ - 13.0 Hz, 6′-H), 4.25 (1H, m, 5-H), 4.35 (1H, d, $J_{3,4}$ 2.8 Hz, 3-H), and 4.62 (1H, dd, $J_{4,3}$ 2.8 Hz and $J_{4,5}$ 7.9 Hz, 4-H); δ_C (67.8 MHz, $CDCl_3$), 23.90 (q), 25.29 (q), 25.72 (q), 26.40 (q), 61.20 (t, C-6), 61.45 (t, C-1), 70.03, 70.74 and 70.94 (3d, C-3, C-4 and C-5), 103.01 (s, C-2), 108.46 (s) and 109.01 (s); m/z (CI) 261 (MH^+, 21%), 245 (MH^+ - CH_4, 58), and 203 (MH^+ - CH_3COCH_3, 100).

2,3 : 4,5 - Di-O-isopropylidene - 1-O-methanesulphonyl-β-D-fructopyranose (229)

To a solution of the primary alcohol (228) (2.00 g, 7.69 mmol) in dry pyridine (25 cm^3) at - 40°C under nitrogen was added dropwise methanesulfonyl chloride (0.72

cm^3, 9.2 mmol). The reaction mixture was allowed to warm to room temperature over 90 min poured into water (200 cm^3) and the resulting aqueous suspension extracted with diethyl ether (3 x 100 cm^3). The combined ethereal extracts were washed with dilute aqueous hydrochloric acid (2 x 100 cm^3), and saturated brine (100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to afford a colourless syrup which crystallised. Recrystallisation from ethyl acetate-light petroleum (b.p. 60-80°C) yield the *mesylate* (229) (1.86 g, 72%) as colourless prisms, m.p. 124-125°C (lit.,[84] 121-122°C) (Found :C, 46.3; H, 6.75. Calc. for $C_{13}H_{22}O_8S$: C, 46.15; H, 6.55%); $[\alpha]_D$ - 27.8° (*c* 1.11 in $CHCl_3$); υ_{max} ($CHCl_3$) 2967 and 2917 (sat. CH), 1365, 1162, 1065, 996 and 962 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.35 (3H, s, C*H*$_3$), 1.42 (3H, s, C*H*$_3$), 1.48 (3H, s, C*H*$_3$), 1.56 (3H, s, C*H*$_3$), 3.07 (3H, s, SO_2CH_3), 3.78 (1H, d, $J_{6,6'}$ - 13.0 Hz, 6-H), 3.92 (1H, dd, $J_{6',5}$ 1.8 Hz and $J_{6',6}$ - 13.0 Hz, 6'-H), 4.22 (1H, d, $J_{1,1'}$ - 11.0 Hz, 1-H), 4.27 (1H, m, 5-H), 4.31 (1H, d, $J_{3,4}$ 2.8 Hz, 3-H), 4.32 (1H, d, $J_{1',1}$ - 11.0 Hz, 1'-H) and 4.63 (1H, dd, $J_{4,3}$ 2.8 Hz and $J_{4,5}$ 8.0 Hz, 4-H); δ_C (67.8 MHz, $CDCl_3$) 23.94 (q), 25.17 (q), 25.79 (q), 26.42 (q), 37.49 (q, SO_2CH_3), 61.37 (t, C-6), 69.64 (t, C-1), 69.83, 70.25, and 70.51 (3d, C-3, C-4, and C-5), 100.7 (s, C-2) and 109.24 (2s); m/z (CI) 339 (MH^+, 21%), 323 (MH^+ - CH_4, 52) and 281 (MH^+ - CH_3COCH_3, 100).

2,3 : 4,5 - Di-O-isopropylidene-1-O-p-toluenesulphonyl-β-D-fructopyran ose (230)

A solution of the primary alcohol (228) (2.00 g, 7.69 mmol) and p-toluenesulphonyl chloride (1.61 g, 8.46 mmol) in dry pyridine (25 cm^3) was stirred for 24h at room temperature under nitrogen. The reaction mixture was poured into water (200 cm^3) and the resulting aqueous suspension extracted with diethyl ether (3 x 100 cm^3). The combined etheral extracts were washed with dilute aqueous hydrochloric acid (2 x 100 cm^3), and saturated brine (100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to afford a colourless syrup. Crystallisation from diethyl ether-light

petroleum (b.p. 60-80°C) on slow cooling to -40°C yielded the *tosylate* (230) (2.26 g, 71%) as colourless needles, m.p. 82°C (lit.,[85] 82°C) (Found C, 55.2; H, 6.2. Calc. for $C_{19}H_{26}O_8S$: C, 55.1; H, 6.3%; $[\alpha]_D$ - 18.2° (*c* 1.02 in $CHCl_3$); υ_{max} ($CHCl_3$) 2964, and 2906 (sat. CH), 1596 (Ar), 1453, 1377 1180, 1105, 1077 and 1012 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.31 (3H, s, C*H*$_3$), 1.36 (6H, s, 2 x C*H*$_3$), 1.50 (3H, s, C*H*$_3$), 2.44 (3H, s, ArC*H*$_3$), 3.70 (1H, dd, $J_{6,5}$ 0.8 Hz and $J_{6,6'}$ - 13.0 Hz, 6-H), 3.86 (1H, dd, $J_{6',5}$ 1.8 Hz and $J_{6',6}$ - 13.0 Hz, 6'-H), 4.00 (1H, d, $J_{1,1'}$ - 10.3 Hz, 1-H), 4.06 (1H, d, $J_{1',1}$ - 10.3 Hz, 1'-H), 4.20 (1H, m, 5-H), 4.30 (1H, d, $J_{3,4}$ 2.8 Hz, 3-H), 4.56 (1H, dd, $J_{4,3}$ 2.8 Hz and $J_{4,5}$ 8.1 Hz, 4-H), 7.34 (2H, m, Ar*H*) and 7.79 (2H, m, Ar*H*); δ (67.8 MHz, $CDCl_3$) 21.50 (q, ArC*H*$_3$), 23.90 (q), 25.07 (q), 25.62 (q), 26.40 (q), 61.20 (t, C-6), 69.05 (t, C-1), 69.80, 69.90, and 70.51 (3d, C-3, C-4 and C-5), 100.58 (s, C-2), 108.95 (s), 109.08 (s), 128.05, and 129.74 (2d, Ar) and 144.86 (s, Ar); m/z (CI) 415 (MH^+, 20%), 399 (MH^+ - CH_4, 70), and 457 (MH^+ - CH_3COCH_3, 100).

2,3 : 4,5-Di-O-isopropylidene-1-O-trifluoromethanesulphonyl-β-D-fructopyranose (236)

To a solution of the primary alcohol (228) (5.0 g, 19.12 mmol) and dry pyridine (10 cm^3) in dry dichloromethane (100 cm^3) at -15°C under nitrogen was added dropwise trifluoromethanesulphonic anhydride (3.88 cm^3, 23.07 mmol). The reaction mixture was stirred for 90 min at -15°C, poured into saturated aqueous sodium bicarbonate (500 cm^3), and the organic layer withdrawn. The aqueous layer was extracted with dichloromethane (2 x 200 cm^3), and the combined organic layers washed with dilute aqueous hydrochloric acid (2 x 100 cm^3), and saturated brine (100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to yield the *triflate* (236) (7.46 g, 99%) as a colourless syrup which solidified, m.p. 38°C (lit.,[88] 36-38.5°C); υ_{max} (film) 2995, and 2943 (sat. CH), 1419, 1386, 1250, 1211, 1146, 1017, and 608 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.36 (3H, s, C*H*$_3$), 1.41 (3H, s, C*H*$_3$), 1.48 (3H, s, C*H*$_3$), 1.57 (3H,

s, C*H*$_3$), 3.80 (1H, br d, $J_{6,6'}$ - 13.0 Hz, 6-H), 3.94 (1H, dd, $J_{6',5}$ 1.9 Hz and $J_{6',6}$ - 13.0 Hz, 6'-H), 4.26 (1H, m, 5-H), 4.32 (1H, d, $J_{3,4}$ 2.8 Hz, 3-H), 4.41 (1H, d, $J_{1,1'}$ - 10.5 Hz, 1-H), 4.53 (1H, d, $J_{1',1}$ - 10.5 Hz, 1' - H) and 4.65 (1H, dd, $J_{4,3}$ 2.8 Hz and $J_{4,5}$ 7.9 Hz, 4-H); m/z (CI) 410 (MNH^+_4, 100%) and 377 (MH^+ - CH_4, 9).

1-Bromo-1-deoxy-2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (231)

A solution of the primary triflate (236) (1.26 g, 3.21 mmol) and potassium bromide (0.42 g, 2.54 mmol) in dry DMF (30 cm^3) was heated at 70°C for 20h under nitrogen. The reaction mixture was poured into diethyl ether (150 cm^3), washed with saturated brine (3 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:9) as the eluant yielded the *bromide* (231) (0.92 g, 89%) as a colourless syrup which crystallised, m.p. 50°C (from light petroleum) (lit.,[84] 50°C) (Found : C, 44.4; H, 5.9. Calc for $C_{12}H_{19}O_5Br$: C, 44.6; H, 5.9%); $[\alpha]_D$ - 32.0° (*c* 0.97 in $CHCl_3$); υ_{max} ($CHCl_3$) 2989, and 2931 (sat. CH), 1379, 1245, 1216, 1167, 1107, 1064, and 997 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.35 (3H, s, C*H*$_3$), 1.46 (3H, s, C*H*$_3$), 1.49 (3H, s, C*H*$_3$), 1.55 (3H, s, C*H*$_3$), 3.51 (1H, d, $J_{1,1'}$ - 11.1 Hz, 1-H), 3.65 (1H, d, $J_{1',1}$ - 11.1 Hz, 1'-H), 3.80 (1H, dd, $J_{6,5}$ 0.7 Hz and $J_{6,6'}$ - 13.0 Hz, 6-H), 3.95 (1H, dd, $J_{6',5}$ 1.83 Hz and $J_{6',6}$ - 13.0 Hz, 6'-H), 4.24 (1H, m, 5-H), 4.36 (1H, d, $J_{3,4}$ 2.8 Hz, 3-H) and 4.62 (1H, dd, $J_{4,3}$ 2.8 Hz and $J_{4,5}$ 7.9 Hz, 4-H); δ_C (67.8 MHz, $CDCl_3$) 23.97 (q), 25.49 (q), 25.82 (q), 26.57 (q), 35.65 (t, C-1), 61.89 (t, C-6), 70.16, 70.61, and 71.45 (3d, C-3, C-4, and C-5), 100.97 (s, C-2), 108.92 (s), and 109.08 (s); m/z (CI) 325 (MH^+ (^{81}Br), 255), 323 (MH^+ (^{79}Br), 29), 309 (MH^+ (^{81}Br)-CH_4, 89) and 307 (MH^+ (^{79}Br)-CH_4, 100).

1-Deoxy-1-iodo-2,3 : 4,5-di-O-isopropylidene-β-D-fructopyranose (232)

(i) A solution of the primary triflate (236) (0.11 g, 0.281 mmol) and potassium bromide (56 mg, 0.337 mmol) in dry DMF (5 cm^3) was heated at 70°C for 24h under nitrogen. The reaction mixture was poured into diethyl ether (50 cm^3), washed with saturated brine (3 x 50 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:9) as the eluant yielded the iodide (232) (103 mg, 99%) as a colourless syrup, identical with the compound described below.

(ii) To a stirred solution of iodine (7 g, 27.6 mmol), triphenylphosphine (7.5 g, 28.6 mmol) and imidazole (4 g, 58.8 mmol) in dry toluene (150 cm^3) was added a solution of the primary alcohol (228) (5.0 g, 19.2 mmol) in dry toluene (50 cm^3) and the mixture refluxed for 16h under nitrogen. The reaction mixture was poured into diethyl ether (500 cm^3), washed with 10% aqueous sodium thiosulphate (2 x 500 cm^3), and saturated brine (2 x 500 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:9) as the eluant yielded the *iodide* (232) (7.00 g, 98%) as a colourless syrup (Found : C, 39.3; H, 5.3 Calc. for $C_{12}H_{19}O_5I$: C, 38.9; H, 5.2%); $[\alpha]_D$ - 19.7° (*c* 0.99 in $CHCl_3$); υ_{max} (film) 2976 and 2918 (sat. CH), 1378, 1247, 1210, 1101, 1064, 978 and 792 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.35 (3H, s, C*H*$_3$), 1.43 (3H, s, C*H*$_3$), 1.48 (3H, s, C*H*$_3$), 1.54 (3H, s, C*H*$_3$), 3.34 (1H, d, $J_{1,1'}$ - 10.6 Hz, 1-H), 3.54 (1H, d, $J_{1',1}$ - 10.6 Hz, 1′-H), 3.79 (1H, br d, $J_{6,6'}$ - 13.2 Hz, 6-H), 3.91 (1H, dd, $J_{6',5}$ 1.8 Hz and $J_{6',6}$ - 13.2 Hz, 6′-H), 4.23 (1H, m, 5-H), 4.31 (1H, d, $J_{3,4}$ 2.6 Hz, 3-H) and 4.59 (1H, dd, $J_{4,3}$ 2.6 Hz and $J_{4,5}$ 7.9 Hz, 4-H); δ_C (67.8 MHz, $CDCl_3$) 10.67 (t, C-1), 24.13 (q), 25.20 (q),

25.95 (q), 26.47 (q), 61.89 (t, C-6), 70.48, 70.61, and 71.75 (3d, C-3, C-4, and C-5), 100.19 (s, C-2), 108.37 (s), and 109.14 (s); m/z (CI) 371 (MH^+, 15%), 355 ($MH^+ - CH_4$, 100) and 313 ($MH^+ - CH_3COCH_3$, 71).

1-Azido-1-deoxy-2,3 : 4,5-di-O-isopropylidene-β-D-fructopyranose (238)

A solution of the primary triflate (236) (5.91 g, 15.08 mmol) and sodium azide (1.08 g, 16.61 mmol) in dry DMF (30 cm^3) was heated at 70°C for 18h under nitrogen. The reaction mixture was poured into diethyl ether (150 cm^3), washed with saturated brine (3 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate - light petroleum (b.p. 60-80°C) (1:9) as the eluant yielded the *azide* (238) (4.25 g, 100%) as a crystalline solid, m.p. 60°C (from light petroleum) (lit.,[91] 55.5 - 60°C) (Found : C, 50.7; H, 6.7; N, 14.7. Calc. for $C_{12}H_{19}O_5N_3$: C, 50.5; H, 6.7; N, 14.7%); $[\alpha]_D$ - 89.9° (*c* 1.02 in $CHCl_3$); υ_{max} ($CHCl_3$) 2992, and 2940 (sat. CH), 2109 (N_3), 1384, 1301, 1252, 1215, 1180, and 1071 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.40 (3H, s, C*H*$_3$), 1.46 (3H, s, C*H*$_3$), 1.49 (3H, s, C*H*$_3$), 1.56 (3H, s, C*H*$_3$), 3.26 (1H, d, $J_{1,1'}$ - 13.0 Hz, 1-H), 3.59 (1H, d, $J_{1',1}$ - 13.0 Hz, 1′-H), 3.76 (1H, br d, $J_{6,6'}$ - 13.0 Hz, 6-H), 3.92 (1H, dd, $J_{6',5}$ 1.9 Hz and $J_{6',6}$ - 13.0 Hz, 6′-H), 4.23 (1H, m, 5-H), 4.29 (1H, d, $J_{3,4}$ 2.6 Hz, 3-H), and 4.61 (1H, dd, $J_{4,3}$ 2.6 Hz and $J_{4,5}$ 7.9 Hz, 4-H); δ_C (67.8 MHz, $CDCl_3$) 23.84 (q), 24.56 (q), 25.69 (q), 26.43 (q), 55.30 (t, C-1), 61.33 (t, C-6), 69.86, and 70.58 (3d, C-3, C-4, and C-5), 102.49 (s, C-2), and 109.01 (2s); m/z (CI) 286 (MH^+, 38%), 270 ($MH^+ - CH_4$, 36), 258 ($MH^+ - N_2$, 25), and 229 ($MH^+ - CH_3COCH_3$, 100).

1-deoxy-1-diethyl phosphoroamidate-2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (239)

A solution of the primary azide (238) (0.459 g, 1.61 mmol) and triethyl phosphate (0.42 cm^3, 2.42 mmol) in dry toluene (10 cm^3) was heated at 70°C for 3h under nitrogen. The reaction mixture was concentrated under reduced pressure and the resulting residue chromatographed on silica gel with dichloromethane - ethanol - ammonia (200:8:1) as the eluant to yield the *phosphoroamidate* (239) (0.57 g, 90%) as a colourless syrup (Found : C, 48.6; H, 7.9; N, 3.4. $C_{16}H_{30}NO_8P$ requires C, 48.6; H, 7.65; N, 3.5%); $[\alpha]_D$ - 17.3° (*c* 0.94 in $CHCl_3$); υ_{max} (film) 3371 (NH), 2988, and 2938 (sat. CH), 1454, 1254, 1070 and 969 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.32 (6H, t, J 7.1 Hz, 2 x CH_2CH_3), 1.35 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.53 (3H, s, CH_3), 3.17 (3H, m, 1-H_2 and N*H*), 3.75 (1H, br d, $J_{6,6'}$ - 13.0 Hz, 6-H), 3.89 (1H, dd, $J_{6',5}$ 1.8 Hz and $J_{6',6}$ - 13.0 Hz, 6'-H), 4.08 (4H, m, 2 x OCH_2CH_3), 4.23 (1H, m, 5-H), 4.29 (1H, d, $J_{3,4}$ 2.6 Hz, 3-H), and 4.59 (1H, dd, $J_{4,3}$ 2.6 Hz and $J_{4,5}$ 8.1 Hz, 4-H); δ_C (67.8 MHz, $CDCl_3$) 15.99 (q), 16.05 (q), 23.84 (q), 25.23 (q), 25.72 (q), 26.34 (q), 47.61 (t, C-1), 61.20 (t, C-6), 62.15 (t), 62.21 (t), 69.99, 70.55, and 71.26 (3d, C-3, C-4. and C-5), 102.53 (d, $J_{C,P}$ 8.8 Hz, C-2), 108.24 (s) and 108.95 (s); m/z (+ve FAB) 396 (MH^+, 100%), 380 (MH^+ - CH_4, 12) and 338 (MH^+ - CH_3COCH_3, 18).

2,3 : 4,5 - Di-O-isopropylidene-1-thiobenzyl-β-D-fructopyranose (243)

To a suspension of 80% sodium hydride dispersion (66 mg, 2.18 mmol) in dry DMF (10 cm^3) at 0°C under nitrogen was added dropwise a solution of benzyl mercaptan (0.26 cm^3, 2.21 mmol) in dry DMF (10 cm^3). After 30 min at 0°C, a solution of the primary triflate (236) (0.777 g, 1.98 mmol) in dry DMF (10 cm^3) was added and the mixture stirred at room temperature for 18h. The reaction mixture was poured into

diethyl ether (150 cm^3), washed with saturated brine (3 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate - light petroleum (b.p. 60-80°C) (1:9) as the eluant yielded the *benzylsulphide* (243) (0.66 g, 96%) as a colourless syrup; υ_{max} (film) 2990, and 2937 (sat. CH), 1383, 1250, 1212, 1168, 1103, 1065, and 993 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.31 (3H, s, C*H*$_3$), 1.40 (3H, s, C*H*$_3$), 1.46 (3H, s, C*H*$_3$), 1.54 (3H, s, C*H*$_3$), 2.67 (1H, d, $J_{1,1'}$ - 16.2 Hz, 1-H), 2.93 (1H, d, $J_{1',1}$ - 16.2 Hz, 1'-H), 3.76 (1H, br d, $J_{6,6'}$ - 12.5 Hz, 6-H), 3.83 (2H, br s, ArC*H*$_2$), 3.93 (1H, dd, $J_{6',5}$ 1.5 Hz and $J_{6',6}$ - 12.5 Hz, 6'-H), 4.21 (1H, m, 5-H), 4.31 (1H, d, $J_{3,4}$ 2.5 Hz, 3-H), 4.58 (1H, dd, $J_{4,3}$ 2.5 Hz and $J_{4,5}$ 7.9 Hz, 4-H) and 7.18-7.48 (5H, m, Ar*H*); m/z (CI) 384 (MNH^+_4, 100%), 367 (MH^+, 23), and 309 (MH^+ - CH_3COCH_3, 7).

2,3 : 4,5-Di-O-isopropylidene-1-thio-β-D-fructopyranose (244)

(i) A solution of the primary triflate (236) (1.226 g, 3.13 mmol) and sodium hydrosulphide (0.35 g, 6.24 mmol) in dry DMF (10 cm^3) was heated at 80°C for 18h under nitrogen. The reaction mixture was poured into diethyl ether (50 cm^3), washed with saturated brine (3 x 50 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl aceate - light petroleum (b.p. 60-80°C) (1:9) as the eluant yielded the *thiol* (244) (0.631 g, 73%) as a colourless syrup which crystallised, m.p. 52°C (from light petroleum) (Found : C, 52.1; H, 7.3%. $C_{12}H_{20}O_5S$ requires C, 52.2; H, 7.3%); $[\alpha]_D$ - 46.5° (*c* 0.96 in $CHCl_3$); υ_{max} ($CHCl_3$) 2991, and 2939 (sat. CH), 1384, 1251, 1214, 1170, 1104, and 1066 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.35 (3H, s, C*H*$_3$), 1.45 (3H, s, C*H*$_3$), 1.48 (3H, s, C*H*$_3$), 1.55 (3H, s, C*H*$_3$), 1.70 (1H, dd, J 7.0 Hz and 10.6 Hz, S*H*), 2.72 (1H, dd, $J_{1,SH}$ 10.6 Hz and $J_{1,1'}$ - 14.1 Hz, 1-H), 3.07 (1H, dd, $J_{1',SH}$ 7.0 Hz and $J_{1',1}$ - 14.1 Hz, 1'-H), 3.78 (1H, br d, $J_{6,6'}$ - 13.0 Hz, 6-H), 3.93 (1H, dd, $J_{6',5}$ 1.8 Hz and $J_{6',6}$ - 13.0 Hz, 6'-H), 4.24

(1H, m, 5-H), 4.33 (1H, d, $J_{3,4}$ 2.8 Hz, 3-H), and 4.61 (1H, dd, $J_{4,3}$ 2.8 Hz and $J_{4,5}$ 8.0 Hz, 4-H); δ_C (67.8 MHz, $CDCl_3$) 23.97 (q), 25.72 (q), 25.82 (q), 26.53 (q), 32.63 (t, C-1), 61.59 (t, C-6), 70.25, 70.74, and 71.81 (3d, C-3, C-4, and C-5), 102.56 (s, C-2), 108.40 (s), and 108.95 (s); m/z (CI) 294 (MNH_4^+, 100%), and 276 (M^+, 16).

(ii) To a solution of the benzyl-sulphide (243) (662 mg, 1.70 mmol) in liquid ammonia (100 cm^3) at -78°C under nitrogen was added sodium metal to maintain a blue colour for 30 min. Excess ammonium chloride (1.0 g) was added and the ammonia allowed to evaporate in a stream of nitrogen. Diethyl ether (100 cm^3) was added, the resulting suspension filtered, and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate - hexane (1:9) as the eluant yielded the *thiol* (244) (468 mg, 96%) as a colourless syrup which crystallised, identical with the sample described above.

1,2 : 4,5-Di-O-isopropylidene-β-D-fructopyranose (227)

To a solution of conc. sulphuric acid (2.5 cm^3) in acetone (555 cm^3) was added dry, finely powdered D-fructose (25.5 g, 0.14 mol), and the resulting suspension stirred, with the exclusion of moisture, at room temperature until the sugar had dissolved (2h). Immediately, an ice-cold solution of sodium hydroxide (2.1 g, 1.1 equiv) in water (100 cm^3) was added gradually. The acetone was removed under reduced pressure, and the resulting aqueous suspension extracted with dichloromethane (3 x 100 cm^3). The combined organic extracts were washed with saturated brine (2 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to afford a crystalline solid. Recrystallisation from diethyl ether - light petroleum (b.p. 60-80°C) yielded the *protected pyranose* (227) (21.90 g, 60%) as colourless needles, m.p. 118°C (lit.,[85] 118-119°C) (Found : C, 55.3; H, 7.9. Calc. for $C_{12}H_{20}O_6$: C, 55.4; H, 7.75%); $[\alpha]_D$ -

143.0° (*c* 1.02 in $CHCl_3$); υ_{max} ($CHCl_3$) 3569 (OH), 2975, and 2927 (sat. CH), 1376, 1117, 1083, and 885 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.37 (3H, s, C*H*$_3$), 1.45 (3H, s, C*H*$_3$), 1.52 (3H, s, C*H*$_3$), 1.54 (3H, s, C*H*$_3$), 1.96 (1H, d, J 8.4 Hz, O*H*), 3.70 (1H, dd, $J_{3,4}$ 6.8 Hz and J 8.4 Hz, 3-H), and 3.97-4.23 (6H, m, 1-H_2, 4-H, 5-H, and 6-H_2), δ_C (67.8 MHz, $CDCl_3$) 25.88 (q), 26.21 (q), 26.37 (q), 27.86 (q), 60.72 (t, C-6), 70.32 (d), 72.26 (t, C-1), 73.27 (d), 77.23 (d), 104.47 (s, C-2), 109.34 (s) and 111.77 (s); m/z (CI) 261 (MH^+, 10%), 245 (MH^+ - CH_4, 33), and 203 (MH^+ - CH_3COCH_3, 100).

1,2-O-isopropylidene-β-D-fructopyranose (264)

To a solution of conc. hydrochloric acid (0.8 cm^3) in water (800 cm^3) was added the diacetonide (227) (15.7 g, 60 mmol) and the resulting solution stirred at room temperature for 24h. The reaction mixture was neutralised with sodium carbonate and the solvent removed under reduced pressure. The residue was suspended in ethyl acetate, dried ($MgSO_4$), and concentrated to afford a crystalline solid. Recrystallisation from ethyl acetate-light petroleum (b.p. 60-80°C) yielded the *mono-acetonide* (264) (10.9 g, 82%) as colourless prisms, m.p. 120°C (lit.,[120] 120-121°C) (Found : C, 49.3; H, 7.6. Calc. for $C_9H_{16}O_6$: C, 49.1; H, 7.3%); $[\alpha]_D$ - 142.6° (*c* 0.77 in $CHCl_3$); υ_{max} ($CHCl_3$) 3547 and 3395 (OH), 2961, 2913 and 2869 (sat. CH), 1375, 1181, 1092, 978 and 887 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.44 (3H, s, C*H*$_3$), 1.50 (3H, s, C*H*$_3$), 2.99 (1H, br s, O*H*), 3.61 (1H, br s, O*H*), 3.77-4.05 (6H, m, 3-H, 4-H, 5-H, 6-H_2, and O*H*), 4.01 (1H, d, $J_{1,1'}$ - 8.8 Hz, 1-H) and 4.19 (1H, d, $J_{1',1}$ - 8.8 Hz, 1′-H); δ_C (67.8 MHz, $CDCl_3$) 25.92 (q), 26.34 (q), 63.77 (t, C-6), 68.28, 68.99 and 71.23 (3d, C-3, C-4 and C-5), 71.42 (t, C-1), 105.61 (s, C-2), and 111.25 (2s); m/z (CI) 221 (MH^+, 4%), 203 (MH^+ - H_2O, 75), and 163 (MH^+ - CH_3COCH_3, 100).

3,4,5-Tri-O-Benzoyl-1,2-O-isopropylidene-β-D-fructopyranose (265)

To a solution of the triol (264) (10.9 g, 49.55 mmol) in dry pyridine (250 cm^3) at room temperature under nitrogen was added dropwise benzoyl chloride (19.0 cm^3, 0.164 mmol). The reaction mixture was heated at 70°C for 8h then cooled. The solution was poured into diethyl ether (1000 cm^3), washed with dilute aqueous hydrochloric acid (2 x 500 cm^3), and saturated brine (500 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate - light petroleum (b.p. 60-80°C) (1:17) as the eluant yielded the *tri-benzoate* (265) (23.20 g, 88%) as a foam, m.p. 52-56°C (Found: C, 67.5; H, 5.5. $C_{30}H_{28}O_9$ requires C, 67.7; H, 5.3%); $[\alpha]_D$ - 293.8° (*c* 1.08 in $CHCl_3$); υ_{max} ($CHCl_3$) 2966 (sat. CH), 1718 (C=O), 1281, 1257, 1106, 1092 and 1071 cm^{-1}; δ_H (270MHz, $CDCl_3$) 1.47 (3H, s, C*H*$_3$), 1.56 (3H, s, C*H*$_3$), 4.07 (1H, dd, $J_{6,5}$ 1.8 Hz and $J_{6,6'}$ - 13.2 Hz, 6-H), 4.09 (1H, d, $J_{1,1'}$ - 9.4 Hz, 1-H), 4.15 (1H, d, $J_{1',1}$ - 9.4 Hz, 1'-H), 4.36 (1H, dd, $J_{6',5}$ 1.3 Hz and $J_{6',6}$ - 13.2 Hz, 6'-H), 5.76 (1H, m, 5-H), 5.86 (1H, dd, $J_{4,5}$ 3.5 Hz and $J_{4,3}$ 10.4 Hz, 4-H), 5.99 (1H, d, $J_{3,4}$ 10.4 Hz, 3-H), and 7.21-8.11 (15H, m, Ar*H*), δ_C (67.8 MHz, $CDCl_3$), 25.96 (q), 26.20 (q), 62.21 (t, C-6), 67.30, 69.76, and 69.93 (3d, C-3, C-4, and C-5), 71.71 (t, C-1), 104.53 (s, C-2), 112.22 (s), 127.95, 128.18, 128.24, 128.73, 129.38, 129.54, 132.82, 133.04, and 133.17 (10d, Ar), 165.28, 165.54, and 165.77 (3s, C=O); m/z (CI) 475 (MH^+ - CH_3COCH_3, 4%), 294 (1) and 203 (5).

3,4,5-Tri-O-benzoyl-β-D-fructopyranose (266)

A solution of the acetonide (265) (23.2 g, 43.61 mmol) in trifluoroacetic acid (200 cm^3) and water (200 cm^3) was stirred for 16h at room temperature. The solvent was removed under reduced pressure, and the resulting residue dissolved in ethyl acetate (400 cm^3). The organic solution was washed with saturated aqueous sodium bicarbonate (2 x 400 cm^3), and saturated brine (400 cm^3), dried ($MgSO_4$), and

concentrated under reduced pressure to afford a crystalline solid. Recrystallisation from ethyl acetate - light petroleum (b.p. 60-80°C) yielded the *diol* (266) (16.09 g, 75%) as colourless needles, m.p. 173-174°C (Found : C, 66.0; H, 4.8. $C_{27}H_{24}O_9$ requires C, 65.85; H, 4.9%); $[\alpha]_D$ - 286.6° (*c* 1.03 in $CHCl_3$); υ_{max} ($CHCl_3$) 3519 (OH), 3013 (unsat. CH), 2919 (sat. CH), 1724 (C=O), 1598, 1447, 1351, 1278, 1103, and 1025 cm^{-1} δ_H (270 MHz, $CDCl_3$) 2.72 (1H, br s, O*H*), 3.58 (1H, br d, $J_{1,1'}$ - 11.7 Hz, 1-H), 3.82 (1H, br d, $J_{1',1}$ - 11.7 Hz, 1'-H), 3.95 (1H, br s, O*H*), 4.04 (1H, dd, $J_{6,5}$ 1.8 Hz and $J_{6,6'}$ - 13.2 Hz, 6-H), 4.40 (1H, dd, $J_{6',5}$ 1.4 Hz and $J_{6',6}$ - 13.2 Hz, 6'-H), 5.75 (1H, m, 5-H), 5.85 (1H, d, $J_{3,4}$ 10.5 Hz, 3-H), 5.97 (1H, dd, $J_{4,5}$ 3.5 Hz and $J_{4,3}$ 10.5 Hz, 4-H), and 7.20-8.10 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 61.56 (t, C-6), 65.49 (t, C-1), 68.41, 69.15 and 70.38 (3d, C-3, C-4, and C-5), 97.43 (s, C-2), 128.25, 128.48, 128.57, 128.83, 129.06, 129.64, 129.81, 129.87, 133.15, 133.37, and 133.54 (11d, Ar), 165.65, 165.84, and 166.36 (3s, C=O), m/z (CI) 475 (MH^+- H_2O, 1%), 353 (2), and 105 (100).

3,4,5-Tri-O-Benzoyl-1-O-p-toluenesulphonyl-β-D-fructopyranose (268)

A solution of the diol (266) (16.0 g, 32.7 mmol) and *p*-toluenesulphonyl chloride (6.86 g, 36.0 mmol) in dry pyridine (250 cm^3) was stirred at room temperature for 48h under nitrogen. The reaction mixture was poured into diethyl ether (1000 cm^3), washed with dilute aqueous hydrochloric acid (2 x 500 cm^3), and saturated brine (500 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (7:13) as the eluant yielded the *primary-tosylate* (268) (20.28 g, 96%) as a foam, m.p. 57-60°C (Found : C, 62.8; H, 4.7. $C_{34}H_{30}O_{11}S$ requires C, 63.15; H, 4.7%); $[\alpha]_D$ - 172.2° (*c* 1.01 in $CHCl_3$); υ_{max} (film) 3434 (OH), 2976 (sat. CH), 1728 (C=O), 1452, 1262, 1178, 1095, and 711 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.36 (3H, s, ArCH_3), 3.64 (1H, br s, O*H*), 4.01 (1H, dd, $J_{6,5}$ 1.8 Hz and $J_{6,6'}$ - 13.2 Hz, 6-H), 4.15 (1H, d, $J_{1,1'}$ -10.6 Hz, 1-H), 4.21 (1H, d, $J_{1',1}$

- 10.6 Hz, 1′-H), 4.36 (1H, dd, $J_{6',5}$ 1.3 Hz and $J_{6',6}$ - 13.2 Hz, 6′-H), 5.72 (1H, m, 5-H), 5.82 (1H, d, $J_{3,4}$ 10.4 Hz, 3-H), 5.90 (1H, dd, $J_{4,5}$ 3.2 Hz and $J_{4,3}$ 10.4 Hz, 4-H), and 7.12-8.08 (19H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 21.54 (q, Ar*C*H_3), 61.63 (t, C-6), 68.24, 69.18, and 70.09 (3d, C-3, C-4, and C-5), 70.45 (t, C-1), 95.98 (s, C-2), 123.93, 125.91, 127.40, 128.02, 128.22, 128.31, 128.51, 128.80, 128.93, 129.61, 129.68, 129.81, 130.16, 132.08, 133.11, 133.34, and 136.45 (17d, Ar), 165.55, and 165.71 (3s, C=O); m/z (+ve FAB) 629 (MH^+ - H_2O, 83%), 554 (41), and 525 (MH^+ - $C_6H_5CO_2H$, 11).

1,2-Anhydro-3,4,5-tri-O-benzoyl-α-D-fructopyranose (269) and
1,2-anhydro-3,4,5-tri-O-benzoyl-β-D-fructopyranose (267)

To a suspension of dry sodium hydride (9 mg, 0.37 mmol) in dry THF (10 cm^3) under nitrogen at 0°C was added dropwise a solution of the primary tosylate (268) (200 mg, 0.31 mmol) in dry THF (5 cm^3). The reaction mixture was stirred for 1h at room temperature, filtered and concentrated under reduced pressure. The resulting residue was dissolved in dry diethyl ether (30 cm^3), filtered through a pad of celite, and concentrated to yield the *spiro-epoxides* (269) and (267) (125 mg, 85%) as a foam with an α : β ratio of 4 : 3, $[\alpha]_D$ - 176.7° (*c* 0.78 in $CHCl_3$); υ_{max} (film) 3070 (unsat. CH), 1729 (C=O), 1287, 1110, 1072, and 708 cm^{-1}; The α- anomer had δ_H (270 MHz, $CDCl_3$) 3.06 (2H, AB, J - 4.8 Hz, 1-H_2), 4.14 (1H, dd, 6-H), 4.42 (1H, dd, 6′-H), 5.59 (1H, d, $J_{3,4}$ 5.7 Hz, 3-H), 5.87 (2H, m, 4-H, and 5-H), and 7.16-8.14 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 50.37 (t, C-1), 63.51 (t, C-6), 66.69, 68.83, and 69.51 (3d, C-3, C-4, and C-5), 81.15 (s, C-2), 128.28-133.70 (m, Ar), 164.77, 165.19, and 165.34 (3s, C=O). The β-anomer had δ_H (270 MHz, $CDCl_3$) 2.90 (1H, d, $J_{1,1'}$ - 3.9 Hz, 1-H), 3.14 (1H, d, $J_{1',1}$ - 3.9 Hz, 1′-H), 4.24 (1H, dd, $J_{6,5}$ 1.5 Hz and $J_{6,6'}$ - 13.3 Hz, 6-H), 4.30 (1H, br d, $J_{6',6}$ - 13.3 Hz, 6′-H), 5.87 (2H, m, 4-H, and 5-H), 6.39 (1H, d, $J_{3,4}$ 9.9 Hz, 3-H), and 7.24-8.14 (15H, m, Ar*H*), δ_C (67.8 MHz, $CDCl_3$) 50.37 (t, C-1), 65.00 (d),

65.55 (t, C-6), 69.73 (d), 70.45 (d), 82.61 (s, C-2), 128.18, 128.28, 128.47, 128.54, 128.80, 129.29, 129.64, 129.84, 133.24, 133.47, and 133.66 (11d, Ar), 165.35, and 165.58 (3s, C=O); m/z (CI) 475 (MH^+, 71%), 465 (58) and 353 (MH^+ - $C_6H_5CO_2H$, 85).

3,4,5-Tri-O-benzoyl-1-deoxy-1-iodo-β-D-fructopyranose (270)

A solution of the primary tosylate (268) (20.28 g, 33.69 mmol) and potassium iodide (5.60 g, 33.70 mmol) in dry DMF (250 cm^3) was heated at 70°C for 24h under nitrogen. The reaction mixture was poured into diethyl ether (1000 cm^3), washed with 10% aqueous sodium thiosulphate (500 cm^3), and saturated brine (2 x 500 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (3:7) as the eluant yielded the *iodohydrin* (270) (17.06 g, 75%) as a foam (Found : C, 54.0; H, 3.9. $C_{27}H_{23}O_8I$ requires C, 53.8; H, 3.85%); $[\alpha]_D$ - 201.3° (*c* 0.99 in $CHCl_3$); υ_{max} (film) 3438 (OH), 3067 (unsat. CH), 2977 (sat. CH), 1728 (C=O), 1602, 1452, 1264, 1092, 1070, and 686 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 3.14 (1H, br s, O*H*), 3.54 (1H, d, $J_{1,1'}$ - 10.8 Hz, 1-H), 3.63 (1H, d, $J_{1',1}$ - 10.8 Hz, 1'-H), 4.09 (1H, dd, $J_{6,5}$ 1.8 Hz and $J_{6,6'}$ - 13.2 Hz, 6-H), 4.39 (1H, dd, $J_{6',5}$ 1.3 Hz, and $J_{6',6}$ - 13.2 Hz, 6'-H), 5.75 (1H, m, 5-H), 5.92 (1H, dd, $J_{4,5}$ 3.3 Hz and $J_{4,3}$ 10.3 Hz, 4-H), 6.03 (1H, d, $J_{3,4}$ 10.3 Hz, 3-H), and 7.21-8.11 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 14.85 (t, C-1), 61.98 (t, C-6), 68.28, 69.80, and 70.12 (3d, C-3, C-4, and C-5), 95.10 (s, C-2), 128.25, 128.51, 129.48, 129.64, 129.84, 129.90, 133.18, 133.41, and 133.63 (9d, Ar), 165.52, and 165.97 (3s, C=O); m/z (+ve FAB) 603 (MH^+, 3%), 585 (MH^+ - H_2O, 26), and 481 (MH^+ - $C_6H_5CO_2H$, 3).

1,2-Anhydro-3,4,5-tri-O-benzoyl-β-D-fructopyranose (267)

To a suspension of silver (I) oxide (19.0 g, 82.0 mmol) in THF (150 cm^3) was added a solution of the iodohydrin (268) (16.32 g, 27.1 mmol) in THF (150 cm^3). The reaction mixture was stirred for 48h at room temperature in the dark, filtered, and concentrated under reduced pressure. The resulting residue was dissolved in dry diethyl ether (150 cm^3), filtered through a pad of celite, and concentrated to yield the *spiro-epoxide* (267) (12.47 g, 97%) as a foam (Found : C, 68.2; H, 4.7. $C_{27}H_{22}O_8$ requires C, 68.35; H, 4.7%); $[\alpha]_D$ - 273.2° (*c* 0.75 in $CHCl_3$); υ_{max} (film) 3069 (unsat. CH), 1729 (C=O), 1283, 1108, 1070, and 708 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.90 (1H, d, $J_{1,1'}$ - 3.9 Hz, 1-H), 3.14 (1H, d, $J_{1',1}$ - 3.9 Hz, 1'-H), 4.24 (1H, dd, $J_{6,5}$ 1.5 Hz and $J_{6,6'}$ - 13.3 Hz, 6-H), 4.30 (1H, br d, $J_{6',6}$ - 13.3 Hz, 6'-H), 5.87 (2H, m, 4-H, and 5-H), 6.39 (1H, d, $J_{3,4}$ 9.9 Hz, 3-H), and 7.24-8.14 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 50.37 (t, C-1), 65.00 (d), 65.55 (t, C-6), 69.73 (d), 70.45 (d), 82.61 (s, C-2), 128.18, 128.28, 128.47, 128.54, 128.80, 129.29, 129.64, 129.84, 133.24, 133.47, and 133.66 (11d, Ar), 165.35, and 165.58 (3s, C=O), m/z (CI) 475 (MH^+, 5%) and 353 (7).

1-azido-1-deoxy-3,4,5-tri-O-benzoyl-β-D-fructopyranose (271)

A solution of the spiro-epoxide (267) (512 mg, 1.08 mmol) and sodium azide (77 mg, 1.19 mmol) in dry DMF (25 cm^3) was stirred at room temperature for 1h under nitrogen. The reaction was quenched by the addition of saturated aqueous ammonium chloride (5 cm^3) and diluted with diethyl ether (150 cm^3). The organic layer was washed with saturated brine (3 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-hexane (3:7) as the eluant yielded the *azide* (271) (212 mg, 38%) as a crystalline solid, m.p. 160-161°C (from diethyl ether-hexane) (Found : C, 62.6; H, 4.3; N, 7.9. $C_{27}H_{23}N_3O_8$ requires C, 62.7; H, 4.5; N, 8.1%); $[\alpha]_D$ - 211.6° (*c* 0.20 in $CHCl_3$); υ_{max} ($CHCl_3$) 3437 (OH),

3068 (unsat. CH), 2110 (N_3), 1728 (C=O), 1602, 1265, 1095, 1070, and 709 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 3.35 (1H, br s, O*H*), 3.35 (1H, d, $J_{1,1'}$ - 11.9 Hz, 1-H), 3.65 (1H, d, $J_{1',1}$ - 11.9 Hz, 1′-H), 4.10 (1H, dd, $J_{6,5}$ 2.0 Hz and $J_{6,6'}$ - 13.1 Hz, 6-H), 4.41 (1H, dd, $J_{6',5}$ 1.6 Hz and $J_{6',6}$ - 13.1 Hz, 6′-H), 5.76 (1H, m, 5-H), 5.88 (1H, d, $J_{3,4}$ 9.8 Hz, 3-H), 5.94 (1H, dd, $J_{4,5}$ 2.7 Hz and $J_{4,3}$ 9.8 Hz, 4-H), and 7.20-8.07 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 56.05 (t, C-1), 61.78 (t, C-6), 68.73, 68.99, and 70.09 (3d, C-3, C-4, and C-5), 97.40 (s, C-2), 128.28, 128.35, 128.51, 128.57, 128.93, 129.64, 129.81, 129.87, 133.21, 133.41, 133.63, and 133.79 (12d, Ar), 165.55, and 165.97 (3s, C=O); m/z (+ve FAB) 500 (MH^+ - H_2O, 32%) and 105 (100).

3,4,5-Tri-O-benzoyl-1-thiobenzyl-β-D-fructopyranose (272)

(i) To a solution of benzyl mercaptan (0.036 cm^3, 0.30 mmol) and DBU (0.46 cm^3) in dry toluene (5 cm^3) under nitrogen was added a solution of the spiro-epoxide (267) (0.132 g, 0.28 mmol) in dry toluene (5 cm^3) and the mixture stirred at room temperature for 1h. The reaction mixture was poured into diethyl ether (50 cm^3), washed with saturated brine (2 x 50 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (2:8) as the eluant yielded the *benzyl sulphide* (272) (92 mg, 55%) as a foam, m.p. 55-60°C (Found : C, 67.8; H, 5.0. $C_{34}H_{30}O_8S$ requires C, 68.2; H, 5.05%); $[\alpha]_D$ - 230.6° (*c* 0.48 in $CHCl_3$); υ_{max} (film) 3439 (OH), 3065, and 3033 (unsat. CH), 2977, and 2870 (sat. CH), 1730 (C=O), 1603, 1266, 1109, and 710 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.60 (1H, d, $J_{1,1'}$ - 14.6 Hz, 1-H), 2.99 (1H, d, $J_{1',1}$ - 14.6 Hz, 1′-H), 3.76 (1H, d, J - 13.1 Hz, ArCH_2), 3.88 (1H, d, J - 13.1 Hz, ArCH_2), 4.03 (1H, dd, $J_{6,5}$ 2.0 Hz and $J_{6,6'}$ - 13.2 Hz, 6-H), 4.11 (1H, br s, O*H*), 4.41 (1H, dd, $J_{6',5}$ 1.3 Hz and $J_{6',6}$ - 13.2 Hz, 6′-H), 5.76 (1H, m, 5-H), 5.85 (1H, d, $J_{3,4}$ 10.4 Hz, 3-H), 5.91 (1H, dd, $J_{4,5}$ 2.9 Hz and $J_{4,3}$ 10.4 Hz, 4-H), and

7.18-8.12 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 37.11 and 38.40 (2t, C-1 and Ar*C*H_2), 61.27 (t, C-6), 69.61, 69.96, and 70.35 (3d, C-3, C-4, and C-5), 97.40 (s, C-2), 127.21, 128.18, 128.38, 128.44, 128.51, 128.90, 129.03, 129.09, 129.16, 129.58, 129.68, 129.77, 133.05, 133.31, 133.41, and 137.46 (16d, Ar), 165.52, 165.71, and 165.97 (3s C=O); m/z (+ve FAB) 581 ($MH^+ - H_2O$, 30%), 461 (25), 391 (43), and 337 (67).

(ii) To a suspension of 80% sodium hydride dispersion (6 mg, 0.20 mmol) in dry DMF (5 cm^3) at 0°C under nitrogen was added dropwise a solution of benzyl mercaptan (0.024 cm^3, 0.20 mmol) in dry DMF (5 cm^3). After 30 min at 0°C, a solution of the spiro-epoxide (267) (87 mg, 0.183 mmol) in dry DMF (5 cm^3) was added and the mixture stirred at room temperature for 30 min. The reaction was quenched by the addition of saturated aqueous ammonium choride (5 cm^3) and diluted with diethyl ether (50 cm^3). The organic layer was washed with saturated brine (2 x 50 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-hexane (2:8) as the eluant yielded the *benzyl sulphide* (272) (50 mg, 46%) identical with the sample described above.

Attempted Arbuzov reaction with 3,4,5-tri-O-benzoyl-1-deoxy-1-iodo-β-D-fructopyranose (267). Formation of 3,4,5-tri-O-benzoyl-1,2-dideoxy-D-fructopyranose (273) and 3,4,5-tri-O-benzoyl-1-deoxy-β-D-fructopyranose (274).

A solution of the iodohydrin (270) (520 mg, 0.864 mmol) in dry trimethyl phosphite (15 cm^3) was heated under reflux for 12h under nitrogen. The solvent was removed under reduced pressure and the residue chromatographed on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:9 to 3:7) as the eluant to yield the *vinyl ether* (273) (79 mg, 20%) as a colourless syrup; υ_{max} (film) 3067 (unsat. CH), 2927

(sat. CH), 1728 (C=O), 1664 (C=C), 1603, 1452, 1263, 1106, 1070, and 710 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 4.10 (1H, dd, $J_{6,5}$ 2.1 Hz and $J_{6,6'}$ - 12.4 Hz, 6-H), 4.37 (1H, dd, $J_{6',5}$ 3.8 Hz and $J_{6',6}$ - 12.4 Hz, 6'-H), 4.70 (1H, m, 1-H), 4.91 (1H, m, 1'-H), 5.67 (1H, dd, $J_{4,5}$ 3.3 Hz and $J_{4,3}$ 9.2 Hz, 4-H), 5.84 (1H, m, 5-H), 6.23 (1H, d, $J_{3,4}$ 9.2 Hz, 3-H), and 7.29-8.09 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 67.79 (d), 68.21 (t, C-6), 68.40 (d), 70.64 (d), 96.92 (t, C-1), 127.79, 127.96, 128.09, 128.51, 128.57, 128.80, 129.19, 129.29, 129.38, 129.64, 129.93, 132.63, 132.85, 132.92, and 133.08 (15d, Ar), 154.09 (s, C-2), 164.74, 164.96, and 165.19 (3s, C=O); m/z (+ve FAB) 459 (MH^+, 40%), 366 (13), and 337 (MH^+ - $C_6H_5CO_2H$, 81).

Further elution gave the *alcohol* (274) (90 mg, 22%) as a crystalline solid, m.p. 150°C (from ethyl acetate-light petroleum) (Found : C, 68.1; H, 4.9. $C_{27}H_{24}O_8$ requires C, 68.1; H, 5.1%); $[\alpha]_D$ - 301.5° (*c* 1.05 in $CHCl_3$); υ_{max} ($CHCl_3$) 3587, and 3341 (OH), 2927 (sat. CH), 1724 (C=O), 1605, 1288, 1261, 1109, and 1092 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.59 (3H, s, C*H*$_3$), 2.83 (1H, br s, O*H*), 4.01 (1H, dd, $J_{6,5}$ 1.9 Hz and $J_{6,6'}$ - 13.2 Hz, 6-H), 4.42 (1H, dd, $J_{6',5}$ 1.3 Hz and $J_{6',6}$ - 13.2 Hz, 6'-H), 5.75 (1H, m, 5-H), 5.88 (2H, m, 3-H and 4-H), and 7.21-8.11 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 26.17 (q, C-1), 61.53 (t, C-6), 69.48, 70.51, and 71.58 (3d, C-3, C-4 and C-5), 97.47 (s, C-2), 128.18, 128.38, 128.48, 129.12, 129.61, 128.77, 133.05, and 133.31 (9d, Ar), 165.68, 165.91 and 166.07 (3s, C=O); m/z (CI) 494 (MNH^+_4, 10%), 372 (6), and 250 (16).

Attempted Michaelis-Becker reaction with 3,4,5-tri-O-benzoyl-1-deoxy-1-iodo-β-D-fructopyranose (267). Formation of 3,4,5-tri-O-benzoyl-1,2-dideoxy-D-fructopyranose (273) and 3,4,5-tri-O-benzoyl-1-deoxy-β-D-fructopyranose (274).

To a suspension of 80% sodium hydride dispersion (8 mg, 0.267 mmol) in dry toluene (4 cm^3) at 0°C under nitrogen was added diethyl phosphite (0.04 ml, 0.310

mmol) and the suspension stirred for 30 min at room temperature. A solution of the iodohydrin (267) (100 mg, 0.166 mmol) in dry toluene (4 cm^3) was added and the reaction mixture refluxed for 3h. The mixture was poured into diethyl ether (40 cm^3), washed with saturated brine (3 x 50 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:9 to 3:7) as the eluant yielded the *vinyl-ether* (273) (8 mg, 10%) as a colourless syrup. Further elution gave the *alcohol* (274) (8 mg, 10%) as an amorphous solid. Both samples were identical with those described above.

3,4,5-Tri-O-benzoyl-1-O-t-butyldimethylsilyl-β-D-fructopyr anose (276)

A solution of the diol (266) (2.475 g, 5.03 mmol), *t*-butyldimethylsilyl chloride (1.14 g, 7.56 mmol) and imidazole (2.40 g, 35.25 mmol) in dry DMF (15 cm^3) was stirred for 18h at room temperature under nitrogen. The reaction mixture was poured into diethyl ether (250 cm^3), washed with saturated brine (3 x 250 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate - hexane (2:8) as the eluant yielded the *silyl-ether* (276) (2.62 g, 86%) as a foam (Found : C, 65.4; H, 6.3. $C_{33}H_{38}O_9S$ requires C, 65.3; H, 6.3%; $[\alpha]_D$ - 224.9° (*c* 0.71 in $CHCl_3$); υ_{max} (film) 3465 (OH), 2932, and 2859, (sat. CH), 1729 (C=O), 1603, 1263, 1108, and 709 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 0.04 (3H, s, SiC*H*$_3$), 0.06 (3H, s, SiC*H*$_3$), 0.86 (9H, s, C(C*H*$_3$)$_3$), 3.68 (1H, d, $J_{1,1'}$ - 10.4 Hz, 1-H), 3.79 (1H, d, $J_{1',1}$ - 10.4 Hz, 1′-H), 3.99 (1H, br s, O*H*), 4.02 (1H, dd, $J_{6,5}$ 1.5 Hz and $J_{6,6'}$ - 11.9 Hz, 6-H), 4.41 (1H, br d, $J_{6',6}$ - 11.9 Hz, 6′-H), 5.76 (1H, m, 5-H), 5.83 (1H, d, $J_{3,4}$ 10.4 Hz, 3-H), 5.96 (1H, dd, $J_{4,5}$ 3.4 Hz and $J_{4,3}$ 10.4 Hz, 4-H), and 7.22-8.10 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) - 5.55 (q), - 5.35 (q), 25.75 (q), 61.37 (t, C-6), 65.68 (t, C-1), 68.53, 69.48, and 70.48 (3d, C-3, C-4, and C-5), 96.88 (s, C-2), 128.22, 128.38, 128.48, 129.03, 129.61, 129.81, 133.05 133.24, and 133.27 (9d, Ar), 165.58, and 166.07 (3s, C=O); m/z (CI) 624 (MNH_4^+, 8%), and 589 (MH^+ - H_2O, 2).

2-O-Acetyl-3,4,5-tri-O-benzoyl-1-O-t-butyldimethylsilyl-α-D-fructopyranose (277α) and 2-O-Acetyl-3,4,5-tri-O-benzoyl-1-O-t-butyldimethylsilyl-β-D-fructopyranose (277β)

A solution of the anomeric alcohol (276) (2.43 g, 4.01 mmol) in dry pyridine (10 cm^3) and dry acetic anhydride (10 cm^3) was heated at 80°C for 3h under nitrogen. The reaction mixture was poured into diethyl ether (200 cm^3), washed with saturated brine (2 x 200 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate - hexane (1:9) as the eluant yielded the *2-O-acetyluloses* (277α) and (277β) (1.35 g, 52%) as a foam with an α : β ratio of 20:3 (Found : C, 64.3; H, 6.1. $C_{35}H_{40}O_{10}S$: requires C, 64.8; H, 6.2%); $[\alpha]_D$ + 38.5° (c 1.20 in $CHCl_3$); υ_{max} (film) 2959, and 2858 (sat. CH), 1728 (C=O), 1257, 1093, 839, and 711 cm^{-1}. The α-anomer had δ_H (270 MHz, $CDCl_3$) 0.17 (3H, s, SiC*H*$_3$), 0.22 (3H, s, SiC*H*$_3$), 1.01 (9H, s, C(C*H*$_3$), 1.93 (3H, s, COC*H*$_3$), 4.32-4.59 (4H, m, 1-H_2 and 6-H_2), 5.86 (1H, m, 5-H), 6.16 (1H, d, $J_{3,4}$ 2 Hz, 3-H), 6.41 (1H, dd, $J_{4,3}$ 2.0Hz and $J_{4,5}$ 8.5 Hz, 4-H), and 7.29-8.06 (15H, m, Ar*H*). The β- anomer had δ_H (270 MHz, $CDCl_3$) 0.07 (3H, s, SiC*H*$_3$), 0.08 (3H, SiC*H*$_3$), 0.88 (9H, s, C(C*H*$_3$)$_3$), 1.96 (3H, s, COC*H*$_3$), 4.32-4.59 (4H, m, 1-H_2 and 6-H_2), 6.11 (2H, m, H-4 and H-5), 6.65 (1H, d, $J_{3,4}$ 9.5 Hz, 3-H), and 7.29-8.06 (15H, m, Ar*H*); m/z (CI) 666 (MNH_4^+, 7%), 544 (18), and 422 (7).

Attempted formation of 3,4,5-tri-O-benzoyl-1-O-t-butyldimethylsilyl-2-deoxy-2-diethylphosphonate-α/β-D-fructopyranose (278)

To a solution of the anomeric acetates (277α) and (277β) (0.314 g, 0.485 mmol; α : β ratio 20:3) and triethyl phosphite (0.13 cm^3, 0.758 mmol) in dry dichloromethane (10 cm^3) at 0°C under nitrogen was added trimethylsilyl trifluoromethanesulphonate (0.11

cm^3, 0.569 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 24h. T.l.c. at this point indicated largely unreacted starting material plus several minor products.

3,4,5-Tri-O-benzoyl-1-deoxy-1-diethylphosphonate-α-D-fructopyranose (284) and 3,4,5-tri-O-benzoyl-1-deoxy-1-diethylphosphonate-β-D-fructopyranose (283).

A solution of the spiro-epoxide (267) (0.157 g, 0.331 mmol) in dry triethyl phosphite (10 cm^3) was heated at 80°C for 2h under nitrogen. The solvent was removed under reduced pressure, and the resulting residue dissolved in diethyl ether (50 cm^3). The organic solution was washed with saturated aqueous sodium bicarbonate (2 x 50 cm^3), and saturated brine (50 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (4:6) as the eluant yielded the *phosphonates* (284) and (283) (0.111 g, 55%) as a foam with an α : β ratio of 1:2 (Found : C, 60.6; H, 5.4. $C_{31}H_{33}O_{11}P$ requires C, 60.8; H, 5.4%); $[\alpha]_D$ - 123.4° (*c* 0.85 in $CHCl_3$); υ_{max} (film) 3300 (OH), 3068 (unsat. CH), 2992, and 2936 (sat. CH), 1727 (C=O), 1601, 1263, 1106, 1030, 760, and 711 cm^{-1}. The α- anomer had δ_H (270 MHz, $CDCl_3$) 1.19-1.30 (6H, m, 2 x OCH_2 CH_3), 2.29-2.46 (2H, m, 1-H_2), 4.00-4.23 (4H, m, 2 x OCH_2CH_3), 4.65-4.87 (2H, m, 6-H_2), 4.49 (1H, m, 5-H), 5.68 (2H, m, 3-H, and 4-H), 6.20 (1H, br s, O*H*), and 7.19-8.14 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 16.07 (dq, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 16.23 (dq, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 31.07 (dt, $J_{C,P}$ 136.6 Hz, C-1), 63.26 (t), 63.83 (t), 78.65, and 80.08 (2d, C-4, and C-5), 82.40 (dd, $J_{C,P}$ 11.0 Hz, C-3), 103.71 (d, $J_{C,P}$ 6.6 Hz, C-2), 128.12-133.57 (m, Ar), 164.84, and 165.94 (2s, C=O). The β-anomer had δ_H (270 MHz, $CDCl_3$) 1.27 (3H, t, 7.1 Hz, OCH_2CH_3), 1.33 (3H, t, J 7.1 Hz, OCH_2CH_3), 2.31 (1H, dd, $J_{1,1'}$ - 14.6 Hz and $J_{1,P}$ 18.5 Hz, 1-H), 2.39 (1H, dd, $J_{1',1}$ - 14.6 Hz and $J_{1',P}$ 18.7 Hz, 1′-H), 4.00-4.23 (5H, m, 6-H and 2 x OCH_2CH_3), 4.50 (1H, dd, $J_{6',5}$ 2.0 Hz and $J_{6',6}$ - 11.9 Hz, 6′-H), 5.75 (1H, m, 5-H), 5.77 (1H, d, $J_{3,4}$ 10.4 Hz, 3-H), 5.95 (1H,

dd, $J_{4,5}$ 3.6 Hz and $J_{4,3}$ 10.4 Hz, 4-H), 6.34 (1H, br s, O*H*), and 7.19-8.12 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 16.07 (dq, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 16.25 (dq, $J_{C,P}$ 4.4 Hz, OCH_2CH_3), 33.60 (dt, $J_{C,P}$ 136.6 Hz, C-1), 61.27 (t, C-6), 61.58 (dt, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 63.17 (dt, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 68.99 (dd, $J_{C,P}$ 4.4 Hz, C-4), 70.51 (d, C-5), 71.83 (dd, $J_{C,P}$ 15.4 Hz, C-3), 96.82 (s, C-2), 128.12, 128.38, 128.44, 128.93, 129.06, 129.51, 129.74, 129.84, 132.95, 133.21, and 133.41 (11d, Ar), 165.35, 165.74, and 166.16 (3s, C=O); m/z (+ve FAB) 613 (MH^+, 3%), 595 ($MH^+ - H_2O$, 19), and 473 (10).

3,4,5-Tri-O-benzoyl-1-deoxy-1-diethylphosphonate-2-O-trimethylsilyl-β-D-fructopyranose (285)

A solution of the spiro-epoxide (283) (3.63 g, 7.66 mmol) in diethyl trimethylsilyl phosphite (5 cm^3) was heated at 90°C for 4h under nitrogen. The reaction mixture was poured into diethyl ether (200 cm^3), washed with saturated aqueous sodium bicarbonate (2 x 200 cm^3), and saturated brine (2 x 200 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (7:13) as the eluant yielded the *phosphonate* (285) (4.36 g, 83%) as a crystalline solid, m.p. 116-117.5°C (from ethyl acetate-light petroleum) (Found : C, 59.6; H, 6.1. $C_{34}H_{41}O_{11}PS$ requires C, 59.6; H, 6.0%); $[\alpha]_D$ - 207.2° (*c* 1.19 in $CHCl_3$); υ_{max} ($CHCl_3$) 2984 (sat. CH), 1729 (C=O), 1603, 1262, 1107, 1025, and 711 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 0.33 (9H, s, $Si(CH_3)_3$), 1.24 (6H, dt, $J_{H,P}$ 2.2 Hz and J 7.1 Hz, 2 x OCH_2CH_3), 2.39 (1H, dd, $J_{1,1'}$ - 15.2 Hz and $J_{1,P}$ 20.6 Hz, 1-H), 2.54 (1H, dd, $J_{1',1}$ - 15.2 Hz and $J_{1',P}$ 18.7 Hz, 1'-H), 4.04 (1H, dd, $J_{6,5}$ 1.6 Hz and $J_{6,6'}$ - 12.1 Hz, 6-H), 4.12 (4H, m, 2 x OCH_2CH_3), 4.26 (1H, br d $J_{6',6}$ - 12.1 Hz, 6'-H), 5.70 (1H, dd, $J_{4,5}$ 3.3 Hz and $J_{4,3}$ 10.1 Hz, 4-H), 5.77 (1H, m, 5-H), 6.41 (1H, d, $J_{3,4}$ 10.1 Hz, 3-H), and 7.21-8.28 (15H, m, ArH); δ_C (67.8 MHz, $CDCl_3$) 1.72 (q, $Si(CH_3)_3$), 16.22 (q, OCH_2CH_3), 16.35 (dq, $J_{C,P}$ 4.5 Hz, OCH_2CH_3), 36.05 (dt, $J_{C,P}$

138.8 Hz, C-1), 61.72 (t, C-6), 62.11 (t, OCH_2CH_3), 62.21 (t, OCH_2CH_3), 69.99, 70.22 and 70.87 (3d, C-3, C-4, and C-5), 98.73 (s, C-2), 128.15, 128.31, 128.38, 129.16, 129.61, 129.71, 130.07, 132.98, and 133.15 (9d, Ar), 165.03, 165.81, and 165.97 (3s, C=O); δ_P (161.8 MHz, $CDCl_3$) + 22.80; m/z (CI) 702 (MNH^+_4, 80%), 685 (MH^+, 41), and 595 (MH^+ - $(CH_3)_3SiOH$, 100).

3,4,5-Tri-O-benzoyl-1-deoxy-1-diethylphosphonate-β-D-fructopyranose (283)

To a solution of the silylether (285) (3.11 g, 4.54 mmol) in dry THF (25 cm^3) at 0°C under nitrogen was added 1M tetrabutylammonium fluoride in THF (5 cm^3, 5 mmol) dropwise. The reaction mixture was stirred at 0°C for 10 min, poured into water (200 cm^3), and the resulting aqueous suspension extracted with diethyl ether (3 x 150 cm^3). The combined ethereal extracts were washed with saturated brine (2 x 200 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (4:6) as the eluant yielded the *alcohol* (283) (2.178 g, 78%) as a foam, m.p. 47.5-52.0°C (Found : C, 60.8; H, 5.4. $C_{31}H_{33}O_{11}P$ requires C, 60.8; H, 5.4%); $[\alpha]_D$ - 201.3° (*c* 1.18 in $CHCl_3$); υ_{max} (film) 3292 (OH), 3068 (unsat. CH), 2985 (sat. CH), 1729 (C=O), 1603, 1266, 1107, 1069, 1026, and 711 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.27 (3H, t, J 7.1 Hz, OCH_2CH_3), 1.33 (3H, t, J 7.1 Hz, OCH_2CH_3), 2.31 (1H, dd, $J_{1,1'}$ - 14.6 Hz and $J_{1,P}$ 18.5 Hz, 1-H), 2.39 (1H, dd, $J_{1',1}$ - 14.6 Hz and $J_{1',P}$ 18.7 Hz, 1'-H), 4.00-4.23 (5H, m, 6-H and 2 x OCH_2CH_3), 4.50 (1H, dd, $J_{6',5}$ 1.0 Hz and $J_{6',6}$ - 11.9 Hz, 6'-H), 5.75 (1H, m, 5-H), 5.77 (1H, d, $J_{3,4}$ 10.4 Hz, 3-H), 5.95 (1H, dd, $J_{4,5}$ 3.6 Hz and $J_{4,3}$ 10.4 Hz, 4-H), 6.34 (1H, br s, O*H*), and 7.19-8.12 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 16.07 (dq, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 16.25 (dq, $J_{C,P}$ 4.4 Hz, OCH_2CH_3), 33.60 (dt, $J_{C,P}$ 136.6 Hz, C-1), 61.27 (t, C-6), 61.58 (dt, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 63.17 (dt, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 68.99 (dd, $J_{C,P}$ 4.4 Hz, C-4), 70.51 (d, C-5), 71.83 (dd, $J_{C,P}$ 15.4 Hz, C-3), 96.82 (s, C-2), 128.12, 128.38, 128.44, 128.93, 129.06, 129.51, 129.74, 129.84,

132.95, 133.21, and 133.41 (11d, Ar), 165.35, 165.74, and 166.16 (3s, C=O); δ_P (161.8 MHz, $CDCl_3$) + 27.55; m/z (CI) 630 (MNH^+_4, 2%), 613 (MH^+, 1) and 595 ($MH^+ - H_2O$, 2).

3,4,5,-Tri-O-benzoyl-1-deoxy-1-diethylphosphonate-2-O-trim ethylsilyl-β-D-fructopyranose (285) and 3,4,5-tri-O-benzoyl-1-deoxy-1-diethyl phosphonate-β-D-fructopyranose (283)

A solution of the spiro-epoxide (267) (515 mg, 1.09 mmol) and catalytic zinc iodide in diethyl trimethylsilyl phosphite (5 cm^3) was heated for 1h at 80°C under nitrogen. The reaction mixture was poured into diethyl ether (75 cm^3), washed with saturated aqueous sodium bicarbonate (2 x 100 cm^3), and saturated brine (2 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-hexane (3:7) as the eluant yielded the *silyl-ether* (285) (394 mg, 53%) as a crystalline solid. Further elution gave the *alcohol* (283) (155 mg, 21%) as a foam. Both samples were identical with those described above.

3,4,5-Tri-O-benzoyl-1-deoxy-1-phosphonic acid β-D-fructopyranose (286)

To a solution of the phosphonate (283) (2.06 g, 3.37 mmol) in dry dichloromethane (50 cm^3) under nitrogen was added bromo trimethylsilane (1.96 cm^3, 14.81 mmol) dropwise. After 24h at room temperature the reaction mixture was concentrated under reduced pressure. The resulting residue was azeotroped with dry THF (3 x 50 cm^3), taken up in THF (25 cm^3), and water (25 cm^3) slowly added. The resulting milky solution was stirred at room temperature for 2h then concentrated. The residue was dissolved in ethyl acetate (100 cm^3), dried ($MgSO_4$), and concentrated to yield the *phosphonic acid* (286) (1.83 g, 98%) as a hygroscopic foam (Found : C, 55.3; H, 4.8. $C_{27}H_{25}O_{11}P$. 1.6 H_2O requires C, 55.4; H, 4.9%); $[\alpha]_D$ - 181.2° (*c* 0.95 in

$CHCl_3$); υ_{max} ($CHCl_3$) 3305 (OH), 1716 (C=O), 1582, 1245, 1180, 1085, and 988 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.40 (2H, m, 1-H_2), 4.00 (1H, br d, $J_{6,6'}$ - 12.5 Hz, 6-H), 4.37 (1H, br d, $J_{6',6}$ - 12.5 Hz, 6'-H), 5.68-5.90 (3H, m, 3-H, 4-H, and 5-H), 7.17-8.06 (15H, m, Ar*H*), and 8.60 (3H, br s, 3 x O*H*); δ_C (67.8 MHz, $CDCl_3$) 34.61 (dt, $J_{C,P}$ 132.2 Hz, C-1), 61.69 (t, C-6), 68.99, and 70.22 (2d, C-4, and C-5), 71.81 (dd, $J_{C,P}$ 13.2 Hz, C-3), 96.93 (d, $J_{C,P}$ 6.6 Hz, C-2), 128.18, 128.41, 128.48, 128.70, 128.93, 129.48, 129.58, 129.81, 129.90, 130.16, 133.08, 133.34, 133.54, and 133.73 (14d, Ar), 165.52, 165.91, and 166.23 (3s, C=O); δ_P (161.8 MHz, $CDCl_3$) + 28.13; m/z (+ve FAB) 557 (MH^+, 83%), and 539 (MH^+ - H_2O, 74).

Dicyclohexyl ammonium 1-deoxy-1-phosphonic acid D-fructose (287)

To a solution of the phosphonic acid (759 mg, 1.37 mmol) in dry methanol (30 cm^3) under nitrogen was added sodium hydroxide (0.54 g, 13.65 mmol) and the reaction mixture stirred at room temperature for 2h. The resulting suspension was concentrated under reduced pressure, dissolved in water (10 cm^3), and passed through a column of Dowex 50 x 8 - 100 (H^+). The combined fractions were concentrated, triturated with ethyl acetate (3 x 10 cm^3), dissolved in water (10 cm^3), washed with ethyl acetate (3 x 10 cm^3) and concentrated. The resulting syrup was dissolved in dry methanol (2 cm^3), dicyclohexylamine (0.24 ml, 1.20 mmol) was added followed by dry acetone (10 cm^3) to precipitate the *phosphonate* (287) (128mg, 22%) as a yellow flocculent solid (Found : C, 51.4; H, 8.6; N, 3.3. $C_{18}H_{36}NO_8P$ requires C, 50.8; H, 8.5; N, 3.3%); $[\alpha]_D$ - 6.0° (*c* 0.22 in MeOH); υ_{max} (nujol mull) 3220 (OH), 1693, 1650, 1608, 1112, 1029, and 898 cm^{-1}; δ_H (270 MHz, D_2O) 1.24-2.31 (12H, m, N(C_6H_5)$_2$ and 1-H_2), 3.19-4.07 (5H, m, 3-H, 4-H, 5-H and 6-H_2), and 4.80 (7H, br d, *H*DO); δ_p (161.8 MHz, D_2O) + 20.11, + 19.48, and + 19.09; m/z (-ve FAB, free acid + 0.1M NaOH (aq)) 287 (M-3H + 2Na, 16%), 265 (M-2H +Na, 5), and 243 (M-H, 7).

2-azido-2-deoxy-3,4,5-tri-O-benzoyl-1-O-trimethylsilyl-β-D-fructopyranose (288)

To a suspension of dry zinc chloride (115 mg, 0.844 mmol) in dry dichloromethane (5 cm^3) under nitrogen was added azidotrimethylsilane (0.12 ml, 0.904 mmol) followed by a solution of the spiro epoxide (267) (207 mg, 0.437 mmol) in dry dichloromethane (10 cm^3) and the reaction mixture stirred for 3h at room temperature. The solution was poured into chloroform (50 cm^3), washed with water (2 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:9 to 1:4) as the eluant yielded the *azide* (288) (151 mg, 61%) as a foam (Found C, 61.3; H, 5.3; N, 6.9. $C_{30}H_{31}O_8N_3Si$ requires C, 61.1; H, 5.3; N, 7.1%); $[\alpha]_D$ - 239.6° (*c* 1.23 in $CHCl_3$); υ_{max} (film) 3066 (unsat. CH), 2962 (sat. CH), 2110 (N_3), 1730 (C=O), 1600, 1257, 1108, 870, 842, and 712 cm^{-1}; δ_H (270 MHz, $CDCl_3$), 0.08 (9H, s, $Si(CH_3)_3$), 3.95 (2H, br s, 1-H_2), 4.22 (1H, dd, $J_{6,5}$ 1.7 Hz and $J_{6,6'}$ - 13.4 Hz, 6-H), 4.34 (1H, dd, $J_{6',5}$ 1.2 Hz and $J_{6',6}$ - 13.4 Hz, 6'-H), 5.74-5.78 (2H, m, 4-H and 5-H), 6.10 (1H, $J_{3,4}$ 10.1 Hz, 3-H), and 7.23-8.11 (15H, m, Ar*H*), m/z (CI) 574 (M^+ - CH_3, 9%), 547 (M^+ -N_3, 13), 516 (M^+ - $Si(CH_3)_3$, 10), and 486 (M^+ - $CH_2 = O^+Si(CH_3)_3$).

2-azido-2-deoxy-1,4,5-tri-O-benzoyl-β-D-fructopyranose (289)

To a solution of the silyl-ether (288) (129 mg, 0.219 mmol) in dry THF (5 cm^3) at 0°C under nitrogen was added 1M tetrabutylammonium fluoride (0.22 ml, 0.22 mmol). The reaction mixture was stirred for 10 min at 0°C, poured into water (25 cm^3) and the resulting aqueous suspension extracted with diethyl ether (3 x 25 cm^3). The combined ethereal extracts were dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:4) as the eluant yielded the *alcohol* (289) (69 mg, 61%) as a glass, m.p. 64-66°C (Found : C, 62.5; H, 4.3; N, 8.0. $C_{27}H_{23}O_8N_3$ requires C, 62.7; H, 4.3; N,

8.1%); $[\alpha]_D$ - 176.6° (*c* 0.94 in $CHCl_3$); υ_{max} ($CHCl_3$) 3552, and 3416 (OH), 3026 (unsat. CH), 2952, and 2920 (sat. CH), 2100 (N_3), 1716 (C=O), 1600, 1584, 1273, 1216, and 1108 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.51 (1H, br s, O*H*), 4.19 (1H, dd, $J_{6,5}$ 1.8 Hz and $J_{6,6'}$ - 13.3 Hz, 6-H), 4.26 (1H, dd, $J_{6',5}$ 1.5 Hz and $J_{6',6}$ - 13.3 Hz, 6′-H), 4.40 (1H, d, $J_{3,4}$ 10.1 Hz, 3-H), 4.78 (1H, d, $J_{1,1'}$ - 11.5 Hz, 1-H), 4.88 (1H, d, $J_{1',1}$ - 11.5 Hz, 1′-H), 5.58 (1H, dd, $J_{4,5}$ 3.3 Hz, and $J_{4,3}$ 10.1 Hz, 4-H), 5.69 (1H, m, 5-H), and 7.26-8.14 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 63.83, and 64.90 (2t, C-1 and C-6), 67.76, 69.67, and 71.19 (3d, C-3, C-4 and C-5), 93.74 (s, C-2), 128.31, 128.51, 129.06, 129.22, 129.42, 129.64, 129.81, 129.90, and 133.37 (9d, Ar), 165.48, 165.97, and 166.39 (3s, C=O); m/z (CI) 475 (M^+ - N_3, 25%), 368 (81), and 311 (80).

4,5-Di-O-benzoyl-2,3,-O-1′-diethyl phosphonate benzylidene-β-D-fructopyranose (292)

To a suspension of dry zinc chloride (0.42 g, 3.08 mmol) in dry dichloromethane (5 cm^3) under nitrogen was added diethyl trimethylsilyl phosphite (0.17 ml, 0.73 mmol) followed by a solution of the spiro-epoxide (267) (290 mg, 0.612 mmol) in dry dichloromethane (10 cm^3) and the reaction mixture stirred for 3h at room temperature. The solution was poured into chloroform (60 cm^3), washed with water (2 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 2:3 to 1:1) as the eluant yielded as the major product the *phosphonate* (292) (176 mg, 47%) as a crystalline solid, m.p. 195.5-196°C (from ethyl acetate-light petroleum) (Found :C, 60.8; H, 5.4 $C_{31}H_{33}O_{11}P$ requires C, 60.8; H, 5.4%); $[\alpha]_D$ - 166.7° (*c* 0.11 in $CHCl_3$); υ_{max} ($CHCl_3$) 3554, and 3416 (OH), 2996 (sat. CH), 1730 (C=O), 1607, 1452, 1262, 1110, and 1018 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.27 (6H, m, 2 x OCH_2CH_3), 1.67 (1H, br s, O*H*), 4.02-4.22 (7H, m, 1-H, 3-H, 6-H and 2 x OCH_2CH_3), 4.31 (1H, br d, $J_{1',1}$ - 8.6 Hz, 1′-H), 4.47 (1H, dd, $J_{6',5}$ 1.1 Hz and $J_{6',6}$ - 13.4 Hz, 6′-H), 5.66-5.72 (2H, m, 4-H

and 5-H), and 7.26-8.09 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 16.38 (q, OCH_2CH_3), 16.48 (q, OCH_2CH_3), 62.93 (t, C-6), 63.94 (dt, $J_{C,P}$ 8.8 Hz, OCH_2CH_3), 64.18 (dt, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 66.98 (d), 70.45 (d), 71.51 (dt, $J_{C,P}$ 6.6 Hz, C-1), 72.30 (d), 108.04 (s, C-2), 109.42 (d, $J_{C,P}$ 205.0 Hz), 126.27, 128.28, 128.48, 129.22, 129.35, 129.61, 129.74, 133.28, 133.37, 136.78, and 137.04 (11d, Ar), and 166.46 (s, C=O); δ_P (161.8 MHz, $CDCl_3$) + 12.32; m/z (CI) 475 (M^+ - $P(O)(OCH_2CH_3)_2$, 12%).

3,4,5-Tri-O-benzyl-1,2-di-O-isopropylidene-β-D-fructopyranose (301)

To a suspension of 60% sodium hydride dispersion (7.21 g, 0.18 mmol) and tetrabutylammonium iodide (6.65 g, 0.018 mol) in dry THF (100 cm^3) at 0°C under nitrogen was added dropwise a solution of the triol (264) (12.01 g, 54.60 mmol) in dry THF (100 cm^3). After 1h at 0°C, benzyl bromide (21.5 cm^3, 0.18 mol) was added dropwise and the mixture stirred at room temperature for 18h. The reaction was quenched by the additon of methanol (10 cm^3), filtered through a pad of celite, and concentrated under reduced pressure. The resulting residue was dissolved in diethyl ether (500 cm^3), washed with saturated brine (2 x 300 cm^3), dried ($MgSO_4$), and concentrated. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (3:17) as the eluant yielded the *tri-benzylether* (301) (21.19 g, 79%) as a colourless syrup which crystallised, m.p. 76-77.5° (diethyl ether-light petroleum) (Found : C, 73.5; H, 6.95%. $C_{30}H_{34}O_6$ requires C, 73.45; H, 7.0%); $[\alpha]_D$ - 81.2° (*c* 0.92 in $CHCl_3$); υ_{max} ($CHCl_3$) 2966, 2912, and 2850 (sat. CH), 1446, 1360, 1108, 1074, 885, and 865 cm^1; δ_H (270 MHz, $CDCl_3$) 1.42 (3H, s, CH_3), 1.47 (3H, s, CH_3), 3.79 (3H, m, 3-H and 6-H_2), 3.92 (2H, m, 4-H and 5-H), 3.98 (2H, AB, J - 8.6 Hz, 1-H_2), 4.62 (2H, AB, J - 11.5 Hz, ArCH_2), 4.66 (1H, d, J - 11.5 Hz, ArCH_2), 4.73 (2H, AB, J - 12.5 Hz, ArCH_2), 5.04 (1H, d, J - 11.5 Hz, ArCH_2), and 7.26-7.41 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 26.11 (q), 27.02 (q), 61.20 (t, C-6), 71.39 (t), 71.78 (t), 71.91 (t), 73.21 (d), 75.09 (d), 75.31 (t), 80.05 (d), 105.80 (s, C-2), 111.71 (s), 127.44,

127.53, 127.60, 127.83, 128.22, and 128.31 (6d, Ar), 138.17, 138.37, and 137.47 (3s, Ar); m/z (CI) 475 (MH^+ - CH_4 25%), 433 (MH^+ - CH_3COCH_3, 66), and 399 (MH^+ - $C_6H_5CH_3$, 41).

3,4,5-Tri-O-benzyl-α/β-D-fructopyranose (302)

A solution of the the acetonide (301) (7.72 g, 15.76 mmol) in trifluoroacetic acid (100 cm^3) and water (100 cm^3) was stirred for 24h at room temperature. The solvent was removed under reduced pressure, and the resulting residue dissolved in diethyl ether (500 cm^3). The organic solution was washed with saturated aqueous sodium bicarbonate (3 x 300 cm^3), and saturated brine (400 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:1) as the eluant yielded the *diol* (302) (3.47 g, 49%) as a colourless syrup with an α : β ratio of 1:2.5; $[\alpha]_D$ - 42.6° (*c* 0.71 in $CHCl_3$); υ_{max} (film) 3444 (OH), 3064, and 3032 (unsat. CH), 2930, and 2878 (sat. CH), 1454, 1084, 1028, 737, and 698 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 3.35-4.04 (9H, m, 1-H_2, 3-H, 4-H, 5-H, 6-H_2 and 2 O*H*), 4.47-5.69 (6H, m, 3 x ArCH_2), and 7.24-7.37 (15H, m, Ar*H*). The α-anomer had δ_C (67.8 Hz, $CDCl_3$) 57.67 (t, C-6), 64.77 (t, C-1), 71.62 (t, ArCH_2), 71.68 (d), 73.40 (t, ArCH_2), 74.15 (d), 74.47 (t, ArCH_2), 97.21 (s, C-2), and 127.67-138.30 (Ar). The β-anomer had δ_C (67.8 MHz, $CDCl_3$) 61.11 (t, C-6), 65.94 (t, C-1), 71.52 (t, ArCH_2), 72.04 (t, ArCH_2), 73.34 (d), 75.57 (t, ArCH_2), 75.74 (d), 79.08 (d), 97.95 (s, C-2), and 127.67-138.30 (Ar); m/z (CI) 433 (MH^+ - H_2O, 30%), and 341 (MH^+ - H_2O - $C_6H_5CH_3$, 22).

3,4,5-Tri-O-benzyl-1-O-p-toluenesulphonyl-α/β-D-fructopyranose (303)

A solution of the diol (302) (3.16 g, 7.02 mmol) and *p*-toluenesulphonyl chloride (1.47 g, 7.72 mmol) in dry pyridine (50 cm^3) was stirred at room temperature for 24h

under nitrogen. The reaction mixture was poured into diethyl ether (500 cm^3), washed with dilute aqueous hydrochloric acid (3 x 250 cm^3), and saturated brine (250 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (7:13) as the eluant yielded the *primary-tosylate* (303) (2.97 g, 70%) as a colourless syrup with an α : β ratio of 1:2.8; $[\alpha]_D$ - 35.0° (*c* 0.42 in $CHCl_3$); υ_{max} (film) 3430 (OH), 3068, and 3038 (unsat. CH), 2934, and 2876 (sat. CH), 1597, 1450, 1364, 1180, and 1089 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.39 (3H, s, ArC*H*$_3$), 3.65-4.19 (7H, m, 1-H_2, 3-H, 4-H, 5-H, and 6-H_2), 4.22-4.96 (6H, m, 3 x ArC*H*$_2$), 7.15-7.40 (17H, m, Ar*H*), and 7.74-7.79 (2H, m, Ar*H*). The α-anomer had δ_C (67.8 MHz, $CDCl_3$) 21.47 (t, ArCH$_3$), 57.34 (t, C-6), 70.35 (t), 71.19 (d), 71.42 (t), 73.43 (t), 73.59 (d), 73.95 (d), 74.08 (t), 96.43 (s, C-2), and 127.60-144.76 (m, Ar). the β-anomer had δ_C (67.8 MHz, $CDCl_3$), 21.47 (t, ArCH$_3$), 60.65 (t, C-6), 70.48 (t), 70.87 (t), 71.62 (t), 72.49 (d), 74.89 (d), 75.35 (t), 78.62 (d), 96.43 (s, C-2), and 127.60-144.76 (m, Ar); m/z (+ve FAB) 601 (MH^+ - H_2O, 22%), 587 (12), and 495 (MH^+ - H_2O -$C_6H_5CH_3$, 93).

Methyl 3,4,5-tri-O-benzyl-β-D-fructopyranose (307)

(i) To a stirred solution of iodine (2.5 g, 9.85 mmol, 1% w/v) in dry methanol (150 cm^3) was added a solution of the acetonide (301) (5.02 g, 10.24 mmol) in dry methanol (100 cm^3) and the mixture refluxed for $4^1/_2$h under nitrogen. The reaction mixture was concentrated under reduced pressure to *ca.* 50 cm^3, poured into diethyl ether (800 cm^3), washed with 10% aqueous sodium thiosulphate (2 x 400 cm^3), and saturated brine (500 cm^3), dried ($MgSO_4$), and concentrated. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (2:3) as the eluant yielded the *methyl-glycoside* (307) (4.52 g, 95%) as a colourless syrup (Found : C, 72.1; H, 7.0; $C_{28}H_{32}O_6$ requires C, 72.4; H, 6.9%; $[\alpha]_D$ - 51.3° (*c* 1.06 in $CHCl_3$); υ_{max} (film) 3476 (OH), 3050,

and 3022 (unsat. CH), 2922, and 2858 (sat. CH), 1457, 1356, 1104, 1068, and 749 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.00 (1H, br s, O*H*), 3.29 (3H, s, OCH_3), 3.53 (1H, dd, $J_{6,5}$ 1.7 Hz and $J_{6,6'}$ - 12.8 Hz, 6-H), 3.69 (2H, br s, 1-H_2), 3.78 (1H, m, 5-H), 3.79 (1H, dd, $J_{6',5}$ 2.0 Hz and $J_{6',6}$ - 12.8 Hz, 6'-H), 4.00 (1H, dd, $J_{4,5}$ 3.1 Hz and $J_{4,3}$ 10.1 Hz, 4-H), 4.21 (1H, d, $J_{3,4}$ 10.1 Hz, 3-H), 4.66 (2H, AB, J - 11.6 Hz, ArCH_2), 4.74 (2H, br s, ArCH_2), 4.75 (1H, d, J - 10.9 Hz, ArCH_2), 5.00 (1H, d, J - 10.9 Hz, ArCH_2), and 7.25-7.41 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 48.88 (q, OCH_3), 61.37 (t, C-6), 63.31 (t, C-1), 71.62 (t, ArCH_2), 72.17 (t, ArCH_2), 73.63 (d), 75.74 (t, ArCH_2), 77.88 (d), 78.91 (d), 99.83 (s, C-2), 127.53, 127.73, 127.86, 128.05, 128.31, 128.38, and 128.70 (7d, Ar), 137.88, 138.30, and 138.40 (3s, Ar); m/z (CI) 482 (MNH_4^+, 100%), 450 (MH^+ - CH_3, 93), and 342 (36).

(ii) To a solution of the acetonide (301) (1.07 g, 2.18 mmol) in dry methanol (75 cm^3) was added *p*-toluenesulphonic acid (0.75 g, 3.94 mmol, 1% w/v) and the mixture refluxed for 24h under nitrogen. The reaction mixture was concentrated under reduced pressure to *ca.* 10 cm^3, poured into diethyl ether (100 cm^3), washed with saturated brine (3 x 100 cm^3), dried ($MgSO_4$), and concentrated. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (2:3) as the eluant yielded the *methyl-glycoside* (307) (0.415 g, 41%) as a colourless syrup identical with the sample described above.

Methyl 3,4,5-tri-O-benzyl-1-deoxy-1-iodo-β-D-fructopyranose (308)

To a stirred solution of iodine (3.13 g, 12.33 mmol), triphenylphosphine (3.46 g, 13.19 mmol) and imidazole (1.80 g, 26.44 mmol) in dry toluene (250 cm^3) was added a solution of the primary-alcohol (307) (4.08 g, 8.79 mmol) in dry toluene (50 cm^3) and the mixture refluxed for 5h under nitrogen. The reaction mixture was poured into

diethyl ether (700 cm^3), washed with 10% aqueous sodium thiosulphate (2 x 300 cm^3), and saturated brine (2 x 300 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light-petroleum (b.p. 60-80°C) (from 3:17 to 1:4) as the eluant yielded the iodide (308) (3.48 g, 69%) as a colourless syrup (Found : C, 58.4; H, 5.4. $C_{28}H_{31}O_5I$ requires C, 58.5; H, 5.4%); $[\alpha]_D$ - 51.7° (*c* 0.68 in $CHCl_3$), υ_{max} (film) 3066, and 3032 (unsat. CH), 2934, and 2878 (sat. CH), 1496, 1452, 1092, 1057, 741, and 701 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 3.24 (3H, s, OCH_3), 3.38 (1H, dd, $J_{6,5}$ 1.0 Hz and $J_{6,6'}$ - 12.6 Hz, 6-H), 3.46 (1H, d, $J_{1,1'}$ - 10.6 Hz, 1-H), 3.56 (1H, d, $J_{1',1}$ - 10.6 Hz, 1'-H), 3.77 (1H, m, 5-H), 3.84 (1H, dd, $J_{6',5}$ 1.9 Hz and $J_{6',6}$ - 12.6 Hz, 6'-H), 3.92 (1H, dd, $J_{4,5}$ 3.2 Hz and $J_{4,3}$ 9.9 Hz, 4-H), 4.51 (1H, d, $J_{3,4}$ 9.9 Hz, 3-H), 4.65 (2H, AB, J - 11.8 Hz, $ArCH_2$), 4.75 (2H, br s, $ArCH_2$), 4.83 (1H, d, J - 11.0 Hz, $ArCH_2$), 5.05 (1H, d, J - 11.0 Hz, $ArCH_2$), and 7.24-7.47 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 4.83 (t, C-1), 48.26 (q, OCH_3), 61.33 (t, C-6), 71.00 (t, $ArCH_2$), 72.04 (t, Ar*C*H), 73.53 (d), 76.12 (t, $ArCH_2$), 77.32 (d), 79.24 (d), 100.09 (s, C-2), 127.44, 127.60, 128.28, and 128.44 (4d, Ar), and 138.43 (s, Ar); m/z (CI) 592 (MNH_4^+, 19%), and 560 (MH^+ - CH_3, 25).

3,4,5-Tri-O-benzyl-1-deoxy-1-iodo-α/β-D-fructopyranose (309)

A solution of the methyl-glycoside (308) (2.68 g, 4.67 mmol) in acetic acid (200 cm^3) and water (48 cm^3) was heated at 100°C for 2h. The reaction mixture was poured into water (400 cm^3) and extracted with dichloromethane (4 x 250 cm^3). The combined organic extracts were washed with saturated aqueous sodium bicarbonate (2 x 500 cm^3), 10% aqueous sodium thiosulphate (250 cm^3), and saturated brine (250 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:4) as the eluant yielded the *alcohol* (309) (2.13 g, 81%) as a colourless syrup with an α : β ratio of 1:4.5 (Found : C, 58.3; H, 5.3 $C_{27}H_{29}O_5I$ requires C, 57.9; H, 5.2%), $[\alpha]_D$ - 30.8 (*c* 1.37 in $CHCl_3$);

υ_{max} (film) 3422 (OH), 3066, and 3032 (unsat. CH), 2926, and 2882 (sat. CH), 1454, 1088, 752, and 694 cm^{-1}. The α-anomer had δ_H (270 MHz, $CDCl_3$) 3.33 (2H, br s, 1-H_2), 3.53-4.05 (5H, m, 3-H, 4-H, 5-H and 6-H_2), 4.40-4.85 (6H, m, 3 x ArCH_2), and 7.17-7.41 (15H, m, Ar*H*). The β-anomer had δ_H (270 MHz, $CDCl_3$) 2.98 (1H, br s, O*H*), 3.30 (1H, d, $J_{1,1'}$ - 10.5 Hz, 1-H), 3.49 (1H, d, $J_{1',1}$ - 10.5 Hz, 1′-H), 3.71-3.76 (3H, m, 5-H and 6-H_2), 3.88 (1H, dd, $J_{4,5}$ 2.9 Hz and $J_{4,3}$ 9.4 Hz, 4-H), 4.14 (1H, d, $J_{3,4}$ 9.4 Hz, 3-H), 4.54-4.77 (5H, m, ArCH_2), 5.02 (1H, d, J 11.2 Hz, ArCH_2), and 7.16-7.41 (15H, m, ArH); m/z (CI) 578 (MNH_4^+, 24%), 560 (M^+, 13), and 450 (M^+ - H_2O - $C_6H_5CH_3$, 100).

1,2-Anhydro-3,4,5-tri-O-benzyl-α-D-fructopyranose (306) and
1,2-anhydro-3,4,5-tri-O-benzyl-β-D-fructopyranose (305)

(i) To a suspension of silver (I) oxide (167 mg, 0.72 mmol) in dry THF (5 cm^3) was added a solution of the iodohydrin (309) (135 mg, 0.24 mmol) in dry THF (5 cm^3). The reaction mixture was stirred for 72h at room temperature in the dark, filtered through a pad of celite, and concentrated under reduced pressure to yield the *spiro-epoxides* (306) and (305) (87 mg, 84%) as a colourless syrup which partially crystallised with an α : β ratio of 1:5; $[\alpha]_D$ - 28.3° (*c* 0.79 in $CHCl_3$); υ_{max} (film) 3058, and 3032 (unsat. CH), 2908, and 2854 (sat. CH), 1451, 1252, 1206, 1102, 1024, 745, and 699 cm^{-1}; The α-anomer had δ_H (270 MHz, $CDCl_3$) 2.81 (2H, AB, J - 5.3 Hz, 1-H_2), 3.57 (1H, dd, $J_{6,5}$ 2.6 Hz and $J_{6,6'}$ - 11.6 Hz, 6-H), 3.78 (1H, dd, J 2.8 Hz and J 6.8 Hz, 4-H), 3.87-3.94 (2H, m, 3-H and 5-H), 4.13 (1H, dd, $J_{6',5}$ 6.2 Hz and $J_{6',6}$ - 11.6 Hz, 6′-H), 4.54-4.60 (6H, m, ArCH_2), and 7.22-7.40 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 49.46 (t, C-1), 64.16 (t, C-6), 71.49 (t, ArCH_2), 72.40 (t, ArCH_2), 73.69, 75.54, and 77.36 (3d, C-3, C-4 and C-5), 82.48 (s, C-2), and 123.97-131.10 (m, Ar). The β-anomer had δ_H (270 MHz, $CDCl_3$) 2.92 (2H, br s, 1-H_2), 3.69 (1H, br d, $J_{6,6'}$

- 11.5 Hz, 6-H), 3.87-3.94 (3H, m, 4-H, 5-H and 6′-H), 4.38 (1H, d, $J_{3,4}$ 9.5 Hz, 3-H), 4.60-4.74 (5H, m, ArC*H*$_2$), 4.91 (1H, d, J - 11.5 Hz, ArC*H*$_2$), and 7.22-7.40 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 50.37 (t, C-1), 64.94 (t, C-6), 71.78 (t, Ar*C*H$_2$), 72.53 (t, Ar*C*H$_2$), 73.14 (d), 73.47 (d), 74.96 (t, Ar*C*H$_2$), 80.05 (d), 83.39 (s, C-2), and 123.97-131.10 (m, Ar); m/z (CI) 433 (MH^+, 26%), 341 (MH^+ - $C_6H_5CH_3$, 86), and 325 (MH^+ - $C_6H_5CH_2OH$, 49).

(ii) To a suspension of potassium *t*-butoxide (0.24 g, 2.14 mmol) in dry THF (15 cm^3) at 0°C under nitrogen was added dropwise a solution of the primary tosylate (303) (1.23 g, 2.04 mmol) in dry THF (15 cm^3). The reaction mixture was stirred for 1h at room temperature, filtered and concentrated under reduced pressure. The resulting residue was dissolved in dry diethyl ether (50 cm^3), filtered through a pad of celite, and concentrated to yield the *spiro-epoxides* (306) and (305) (0.765 g, 87%) as a colourless syrup which partially crystallised with an α : β ratio of 1:6.

3,4,5-Tri-O-benzyl-1-deoxy-1-diethylphosphonate-2-O-trimethylsilyl-β-D-fructopyranose (310)

A solution of the spiro-epoxides (305) and (306) (122 mg, 0.282 mmol) in diethyl trimethylsilyl phosphite (3 cm^3) was heated at 100°C for 3h under nitrogen. The reaction mixture was poured into diethyl ether (50 cm^3), washed with saturated aqueous sodium bicarbonate (2 x 50 cm^3), and saturated brine (2 x 50 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (3:7) as the eluant yielded the *phosphonate* (310) (92 mg,50%) as a colourless syrup; δ_H (270 MHz, $CDCl_3$) 0.09 (9H s, Si(C*H*$_3$)$_3$), 1.24 (6H, q, J 7.2 Hz, 2 x OCH$_2$C*H*$_3$), 2.22 (1H, dd, $J_{1,1'}$ - 15.0 Hz and $J_{1,P}$ 20.3 Hz, 1-H), 2.58 (1H, dd, $J_{1',1}$ - 15.0 Hz and $J_{1',P}$ 20.0 Hz, 1′-H), 3.66-3.84

(4H, m, 4-H, 5-H and 6-H_2), 4.07 (4H,m, 2 x O*C*H_2CH$_3$), 4.41 (1H, d, $J_{3,4}$ 9.5 Hz, 3-H), 4.62 (2H, br s, ArCH_2), 4.69 (2H, AB, J - 13.0 Hz, ArCH_2), 4.86 (1H, d, J - 11.2 Hz, ArCH_2), 5.04 (1H, d, J - 11.2 Hz, ArCH_2), and 7.25-7.41 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 1.95 (q, Si(*C*H$_3$)$_3$), 16.31 (q), 16.38 (q), 36.07 (dt, $J_{C,P}$ 136.6 Hz, C-1), 60.98 (t, C-6), 61.35 (dt, $J_{C,P}$ 6.6 Hz, O*C*H_2CH$_3$), 62.06 (dt, $J_{C,P}$ 6.6 Hz, O*C*H_2CH$_3$), 71.39 (t, Ar*C*H_2), 71.78 (t, Ar*C*H_2), 73.76 (d), 75.22 (t, Ar*C*H_2), 78.46 (d), 78.78 (d), 99.58 (s, C-2), 127.21, 127.44, 127.66, 127.73, 128.02, 128.09, and 128.25 (7d, Ar), 138.53, and 139.11 (2s, Ar).

3,4,5-Tri-O-benzyl-1-deoxy-1-diethylphosphonate-β-D-fructopyranose (311)

To a solution of the phosphonate (310) (63 mg, 0.098 mmol) in dry THF (5 cm^3) at 0°C under nitrogen was added 1M tetrabutylammonium fluoride in THF (0.11 cm^3, 0.11 mmol) dropwise. The reaction mixture was stirred at 0°C for 10 min, poured into water (30 cm^3) and the resulting aqueous supension extracted with diethyl ether (3 x 20 cm^3). The combined ethereal extracts were washed with saturated brine (2 x 50 cm^3), dried ($MgSO_4$), and concentrated under reduced presssure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:1) as the eluant yielded the *alcohol* (310) (34 mg, 61%) as a colourless syrup; $[\alpha]_D$ - 25.9° (*c* 0.33 in $CHCl_3$); υ_{max} (film) 3280 (OH), 2930, and 2876 (sat. CH), 1409, 1171, 1059, 1018, 988 and 660 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.28 (6H, t, J 7.1 Hz, 2 x OCH$_2$C*H*$_3$), 1.83 (1H, dd, $J_{1,1'}$ - 15.1 Hz and $J_{1,P}$ 18.6 Hz, 1-H), 2.39 (1H, dd, $J_{1',1}$ - 15.1 Hz and $J_{1',P}$ 17.4 Hz, 1'-H), 3.68 (1H, dd, $J_{6,5}$ 1.8 Hz and $J_{6,6'}$ - 12.5 Hz, 6-H), 3.74 (1H, d, $J_{3,4}$ 10.8 Hz, 3-H), 3.78 (1H, m, 5-H), 3.89 (1H, dd, $J_{6',5}$ 1.5 Hz and $J_{6',6}$ - 12.5 Hz, 6'-H), 3.87-4.50 (5H, m, 4-H and 2 x O*C*H_2CH$_3$), 4.66 (2H, AB, J - 11.9 Hz, ArCH_2), 4.68 (1H, d, J - 11.5 Hz, ArCH_2), 4.75 (2H, br s, ArCH_2), 5.03 (1H, d, J - 11.5 Hz, ArCH_2), 5.80 (1H, br s, O*H*), and 7.26-7.40 (15H, m, Ar*H*), δ_C (67.8 MHz, $CDCl_3$) 16.28 (q, OCH$_2$*C*H$_3$), 16.35 (q, OCH$_2$*C*H$_3$), 33.28 (dt, $J_{C,P}$ 136.6 Hz, C-1), 60.88 (t, C-6), 61.46

(dt, $J_{C,P}$ 4.4 Hz, OCH_2CH_3), 62.71 (dt, $J_{C,P}$ 6.6 Hz, OCH_2CH_3), 71.62 (t, $ArCH_2$), 72.26 (t, $ArCH_2$), 73.89 (d), 75.28 (t, $ArCH_2$), 78.69 (d), 79.34 (dd, $J_{C,P}$ 13.2 Hz, C-3), 97.45 (d, $J_{C,P}$ 6.6 Hz, C-2), 127.60, 127.66, 127.73, 127.83, 128.03, 128.12, 128.35, 128.60, 128.99, 129.74, 129.84, and 130.06 (12d, Ar), 133.15, 138.30, and 138.47 (3s, Ar); m/z (-ve FAB) 569 (M-1, 100%), 541 (M-1-CH_2=CH_2, 25), and 507 (11).

2,3,5-Tri-O-benzyl-α/β-D-arabinose (315)[145]

To a solution of Drierite (5 g) and sulphuric acid (1.5 cm^3) in dry methanol (200 cm^3) under nitrogen was added D-arabinose (10.0 g, 66.61 mmol) and the reaction mixture stirred at room temperature for 15h. The mixture was filtered through a pad of celite, amberlite resin IRA- 93 (OH) added and the suspension stirred for 2h until the solution became neutral. The suspension was filtered, concentrated under reduced pressure and azeotroped with dry THF (3 x 100 cm^3). The resulting syrup was dissolved in dry THF (70 cm^3), drierite (5 g), powdered potassium hydroxide (26 g, 0.46 mol), and benzoyl chloride (30 cm^3, 0.29 mol) added, and the mixture refluxed for 48h under nitrogen. The suspension was filtered through a bed of celite and concentrated under reduced pressure finally at *ca.* 1 mmHg and 130°C. The resulting crude methyl 2,3,5-tri-O-benzyl-D-arabinoside (314) was dissolved in glacial acetic acid (400 cm^3), the solution diluted with 6M aqueous hydrochloric acid (60 cm^3), and the reaction mixture heated at 80°C for 75 min. The mixture was concentrated under reduced pressure to *ca.* 100 cm^3, diluted with water (1000 cm^3), and the resulting aqueous suspension extracted with ether (3 x 500 cm^3). The combined etheral extracts were washed with saturated aqueous sodium bicarbonate (3 x 750 cm^3), and saturated brine (750 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:3 to 3:7) yielded the *alcohol* (315) (8.95 g, 32%) as a crystalline solid of undetermined anomeric configuration, m.p. 72-74°C (from diethyl ether-light

petroleum) (Found : C, 74.1; H, 6.7. Calc. for $C_{26}H_{28}O_5$. C, 74.3; H, 6.7%); $[\alpha]_D$ - 2.8° (*c* 1.17 in $CHCl_3$); υ_{max} ($CHCl_3$) 3518, and 3396 (OH), 2864, and 2804 (sat. CH), 1428, 1328, and 1049 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 3.40 (1H, br s, O*H*), 3.47-3.62 (2H, m, 5-H_2), 3.92-4.16 (3H, m, 2-H, 3-H, and 4-H), 4.45-4.67 (6H, m, 3 x ArCH_2), 5.30-5.39 (1H, m, 1-H), and 7.22-7.33 (15H, m, Ar*H*); m/z (CI) 403 (MH^+ - H_2O, 11%), 329 (MH^+ - $C_6H_5CH_3$, 12), 311 (MH^+ - H_2O - $C_6H_5CH_3$, 35), and 295 (MH^+ - H_2O -$C_6H_5CH_2OH$, 89).

2,3,5-Tri-O-benzyl-D-arabino-1,4-lactone (316)

To a suspension of pyridinium chlorochromate (2.00 g, 9.28 mmol) in dry dichloromethane (20 cm^3) under nitrogen was added a solution of the alcohol (315) (2.60 g, 6.19 mmol) in dry dichloromethane (30 cm^3) and the reaction mixture stirred for 72h. The resulting suspension was diluted with diethyl ether (50 cm^3), filtered through a pad of celite, and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (3:17) as the eluant yielded the *lactone* (316) (2.15 g, 83%) as a crystalline solid, m.p. 66°C (from diethyl ether-light petroleum (lit.,[146] 67°C) (Found : C, 74.2; H, 6.2. Calc. for $C_{26}H_{26}O_5$; C, 74.6; H, 6.3%); $[\alpha]_D$ + 6.5° (*c* 1.13 in $CHCl_3$); υ_{max} ($CHCl_3$) 3011 (unsat. CH), 2815 (sat. CH), 1805 (lactone), 1432, 1336, 1291, and 1084 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 3.58 (1H, br d, $J_{5,5'}$ - 11.0 Hz, 5-H), 3.71 (1H, br d, $J_{5',5}$ - 11.0 Hz, 5'-H), 4.34 (3H, m, 2-H, 3-H, and 4-H), 4.50 (1H, d, J - 11.9 Hz, ArCH_2), 4.54 (2H, AB, J - 11.6 Hz, ArCH_2), 4.63 (1H, d, J - 11.9 Hz, ArCH_2), 4.77 (1H, d, J - 11.5 Hz, ArCH_2), 5.07 (1H, d, J - 11.5 Hz, ArCH_2), and 7.20-7.41 (15H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 67.82 (t, C-5), 72.36, 72.56, and 73.34 (3t, $ArCH_2$), 78.69, 79.01, and 79.08 (3d, C-2, C-3 and C-4), 127.60, 127.73, 127.79, 127.99, 128.09, 128.31, 128.38, and 128.44 (8d, Ar), 136.68, 136.97, and 137.36 (3s, Ar), and 172.38 (s, C-1); m/z (CI) 327 (MH^+ - $C_6H_5CH_3$, 5%), and 311 (MH^+ - $C_6H_5CH_2OH$, 1).

Attempted Wittig reaction with 2,3,5-tri-O-benzyl-D-arabino-1,4-lactone (316). Formation of 2,6-di-O-benzyl-3-deoxy-D-glycero-pent-2-eno-1,4-lactone (317)

To a suspension of 60% sodium hydride dispersion (14 mg, 0.353 mmol) in dry DMSO ($1^1/_2$ cm^3) at 0°C under nitrogen was added methyltriphenylphosphonium iodide (146 mg, 0.360 mmol) and the suspension stirred for 1h to afford a homogeneous yellow solution. A solution of the lactone (316) (134 mg, 0.321 mmol) in dry DMSO ($3^1/_2$ cm^3) was added and the reaction mixture stirred at room temperature for 20h. The mixture was poured into water (50 cm^3) and extracted with diethyl ether (3 x 50 cm^3). The combined ethereal extracts were dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. (60-80°C) (1:4) as the eluant yielded the α,β-*unsaturated lactone* (317) (22 mg, 22%) as a colourless syrup; υ_{max} (film) 2895, and 2823 (sat. CH), 1761 (C=O), 1635 (C=C), 1430, 1099, 719, and 679 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 3.59 (1H, dd, $J_{5,4}$ 5.0 Hz and $J_{5,5'}$ - 10.4 Hz, 5-H), 3.63 (1H, dd, $J_{5',4}$ 5.3 Hz and $J_{5',5}$ - 10.4 Hz, 5'-H), 4.55 (2H, AB, J - 12.1 Hz, ArCH_2), 5.01 (2H, AB, J - 11.8 Hz, ArCH_2), 5.04 (1H, m, 4-H), 6.08 (1H, d, $J_{3,4}$ 2.0 Hz, 3-H), and 7.26-7.39 (10H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 70.58 (t, C-5), 72.88 (t, ArCH_2), 73.69 (t, ArCH_2), 77.65 (d, C-4), 115.31 (d, C-3), 127.63, 127.73, 127.96, 128.51, 128.57, and 128.67 (6d, Ar), 134.64, and 137.33 (2s, Ar), and 146.48 (s, C-1); m/z (+ve FAB) 311 (MH^+, 82%), 279 (54), and 271 (21).

Attempted epoxidation of 2,3,5-tri-O-benzyl-D-arabino-1,4-lactone (316). Formation of 2,6-di-O-benzyl-3-deoxy-D-glycero-pent-2-eno-1,4-lactone (317).

To a suspension of 60% sodium hydride dispersion (11 mg, 0.274 mmol) in dry DMSO (2 cm^3) at 0°C under nitrogen was added trimethylsulphoxonium iodide (61 mg, 0.275 mmol) and the suspension stirred for 15 min to afford a homogeneous

solution. A solution of the lactone (316) (104 mg, 0.249 mmol) in dry DMSO (3 cm^3) was added and the reaction mixture stirred at room temperature for 12h. The mixture was poured into water (50 cm^3) and extracted with diethyl ether (3 x 50 cm^3). The combined ethereal extracts were dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:4) as the eluant yielded the α,β-*unsaturated lactone* (317) (4 mg, 5%) as a colourless syrup identical with the sample described above.

Methyl 1,6-di-O-p-toluenesulphonyl-β-D-fructofuranose (328) and methyl 1,6-di-O-p-toluenesulphonyl-α-D-fructofuranose (329).

To a solution of conc. sulphuric acid (0.8 ml) in dry methanol (200 cm^3) under nitrogen was added D-fructose (5.04 g, 27.98 mmol) and the resulting solution stirred for 30 min at room temperature. Amberlite resin IRA - 93 (OH) was added and stirring continued for 2h until the solution became neutral. The suspension was filtered, concentrated under reduced pressure and azeotroped with dry pyridine (3 x 50 cm^3). The resulting syrup was dissolved in dry pyridine (75 cm^3) and *p*-toluenesulphonyl chloride (10.67 g, 55.97 mmol) added at 0°C under nitrogen. After 108h at 4°C and 48h at room temperature the solution was poured into water (800 cm^3) and the resulting aqueous suspension extracted with chloroform (3 x 500 cm^3). The combined organic extracts were dried ($MgSO_4$), concentrated under reduced pressure and azeotroped with dry toluene (2 x 250 cm^3). Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:1 to 3:2) as the eluant yielded the α-*glycoside* (329) (1.12 g, 8%) as a colourless syrup (Found : C, 50.6; H, 5.3 Calc. for $C_{21}H_{26}O_{10}S_2$: C, 50.2; H, 5.2%); $[\alpha]_D$ + 21.5° (*c* 1.06 in $CHCl_3$); υ_{max} ($CHCl_3$) 3588, and 3392 (OH), 2874 (sat. CH), 1594, 1324, 1105, 1017, 908, and 735 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.44 (3H, s, ArCH_3), 2.45 (3H, s, ArCH_3), 3.11 (2H, br s, 2 x O*H*), 3.16 (3H, s, OCH_3), 3.85 (1H, m, 5-H), 4.05-4.11 (6H, m,

1-H_2, 3-H, 4-H and 6-H_2), 7.32-7.38 (4H, m, Ar*H*), and 7.75-7.81 (4H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 21.57 (q, Ar*C*H_3), 21.63 (q, Ar*C*H_3), 48.72 (q, O*C*H_3), 62.89, and 69.28 (2t, C-1 and C-6), 78.17, 79.24, and 83.81 (3d, C-3, C-4 and C-5), 107.91 (s, C-2), 127.92, 128.15, 129.14 and 129.97 (4d, Ar), 131.85, 132.34, 145.11, and 145.41 (4s, Ar); m/z (+ve FAB) 471 (MH^+ - CH_3OH, 95%), 453 (5), and 391 (5). Further elution gave the β-*glycoside* (328) (2.81 g, 20%) as a crystalline solid, m.p. 113.5-115.5°C (from ethyl acetate-light petroleum) (lit.,[159] 109-110°C) (Found : C, 50.4; H, 5.4. Calc. for $C_{21}H_{26}O_{10}S_2$: C, 50.2; H, 5.2%); $[\alpha]_D$ - 13.3° (*c* 0.97 in $CHCl_3$); υ_{max} ($CHCl_3$) 3538, and 3352 (OH), 2914 (sat. CH), 1593, 1362, 1168, 1093, 984, and 827 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.45 (6H, s, 2 x Ar*C*H_3), 2.58 (2H, br s, 2 x O*H*), 3.19 (3H, s, O*C*H_3), 3.87 (1H, m, 5-H), 3.99-4.14 (6H, m, 1-H_2, 3-H, 4-H and 6-H_2), and 7.76-7.80 (4H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$), 21.63 (q, Ar*C*H_3), 49.27 (q, O*C*H_3), 66.23 and 69.02 (2t, C-1 and C-6), 75.35, and 78.56 (3d, C-3, C-4 and C-5), 101.23 (s, C-2), 127.89, 127.96, 129.93, and 132.11 (4d, Ar), 145.18, and 145.31 (2s, Ar); m/z (CI) 520 (MNH_4^+, 2%), 348 (5), and 316 (8).

Methyl 3,4-di-O-benzyl-1,6-di-O-p-toluenesulphonyl-β-D-fructofuranose (330) and methyl 1,4-anhydro-3-O-benzyl-6-O-p-toluenesulphonyl-β-D-fructofuranose (331).

To a suspension of 60% sodium hydride dispersion (41 mg, 1.03 mmol) and tetrabutylammonium iodide (37 mg, 0.1 mmol) in dry THF (5 cm^3) at 0°C under nitrogen was added dropwise a solution of the diol (328) (230 mg, 0.458 mmol) in dry THF (5 cm^3). After 30 min at 0°C, benzyl bromide (0.14 ml, 1.18 mmol) was added dropwise and the mixture stirred at room temperature for 48h. The reaction was quenched by the addition of methanol (1 cm^3), poured into diethyl ether (100 cm^3), washed with saturated brine (3 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:3 to 2:3) as the eluant yielded the *di-benzyl ether* (330) (43

mg, 14%) as a colourless syrup; δ_H (270 MHz, $CDCl_3$) 2.41 (6H, s, 2 x ArCH_3), 3.18 (3H, s, OCH_3), 3.90 (1H, m, 5-H), 3.96-4.11 (6H, m, 1-H_2, 3-H, 4-H and 6-H_2), 4.39 (1H, d, J - 11.5 Hz, ArCH_2), 4.50 (2H, AB, J - 9.7 Hz, ArCH_2), 4.63 (1H, d, J - 11.5 Hz, ArCH_2), 7.16-7.33 (14H, m, Ar*H*), and 7.72-7.76 (4H, m, Ar*H*). Further elution gave the *anhydro-sugar* (331) (38 mg, 20%) as a colourless amorphous solid; υ_{max} ($CHCl_3$) 2936 and 2872 (sat. CH), 1598, 1449, 1359, 1167, 1052, 983, and 889 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.41 (3H, s, ArCH_3), 3.34 (3H, s, OCH_3), 3.87 (1H, dd, $J_{6,5}$ 1.4 Hz and $J_{6,6'}$ - 8.7 Hz, 6-H), 3.91 (1H, d, $J_{6',6}$ - 8.7 Hz, 6′-H), 4.16 (1H, d, $J_{1,1'}$ - 2.2 Hz, 1-H), 4.17 (1H, d, $J_{3,4}$ 11.3 Hz, 3-H), 4.21 (1H, d, $J_{1',1}$ - 2.2 Hz, 1′-H), 4.30 (1H, m, 5-H), 4.50 (1H, br d, $J_{4,3}$ 11.3 Hz, 4-H), 4.53 (2H, AB, J - 11.7 Hz, ArCH_2), 7.24-7.39 (7H, m, Ar*H*), and 7.74 (2H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$), 21.60 (q, ArCH_3), 50.05 (q, OCH_3), 64.42, 71.16, and 72.36 (3t, C-1, C-6 and ArCH_2), 76.00, 78.65, and 83.68 (3d, C-3, C-4 and C-5), 105.71 (s, C-2), 127.67, 127.92, 128.05, 128.54, and 129.77 (5d, Ar), 136.75, and 144.86 (2s, Ar); m/z (-ve FAB) 419 (M-1, 64%), 375 (18), and 352 (30).

Methyl 3,4-di-O-benzoyl-1,6-di-O-p-toluenesulphonyl-β-D-fructofuranose (332)

To a solution of the diol (328) (1.50 g, 2.99 mmol) in dry pyridine (25 cm^3) at room temperature was added dropwise benzoyl chloride (0.76 cm^3, 6.55 mmol) and the reaction mixture heated at 80°C for $2^1/_2$h under nitrogen. The solution was poured into diethyl ether (500 cm^3), washed with dilute aqueous hydrochloric acid (3 x 500 cm^3), and saturated brine (500 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:3 to 3:7) as the eluant yielded the *di-benzoate* (332) (1.56 g, 73%) as a colourless syrup (Found : C, 59.4; H, 5.2. $C_{35}H_{34}O_{12}S_2$ requires C, 59.15; H, 4.8%); $[\alpha]_D$ - 43.0° (*c* 1.26 in $CHCl_3$); υ_{max} (film) 3072, and 3036 (unsat. CH), 2962 (sat. CH), 1730 (C=O), 1604, 1490, and 1450 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.37 (3H,

s, ArCH_3), 2.39 (3H, s, ArCH_3), 3.28 (3H, s, OCH_3), 4.17-4.26 (4H, m, 1-H_2 and 6-H_2), 4.44 (1H, m, 5-H), 5.63 (1H, m, 4-H), 5.79 (1H, d, $J_{3,4}$ 7.7 Hz, 3-H), and 7.25-7.99 (18H, m, ArH); δ_C (67.8 MHz, $CDCl_3$), 21.57 (q, ArCH_3), 50.11 (q, OCH_3), 67.66 (t, C-6), 69.12 (t, C-1), 75.64, 76.51, and 78.07 (3d, C-3, C-4, and C-5), 102.53 (s, C-2), 127.99, 128.38, 128.48, 128.83, 129.77, 129.87, 132.34, 132.50, 133.41, and 133.66 (10d, Ar), 145.02 (s, Ar), and 165.13 (s, C=O); m/z (CI) 728 (MNH^+_4, 1%), 574 (0.5), and 556 (0.5).

Methyl 3,4-di-O-benzoyl-6-deoxy-6-iodo-1-O-p-toluenesulphonyl-β-D-fructofuranose (333)

A solution of the di-tosylate (332) (1.12 g, 1.58 mmol) and potassium iodide (270 mg, 1.63 mmol) in dry DMF (25 cm^3) was heated at 110°C for 36h under nitrogen. The reaction mixture was poured into diethyl ether (200 cm^3), washed with 10% aqueous sodium thiosulphate (150 cm^3), and saturated brine (2 x 200 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p.60-80°C) (3:17) as the eluant yielded the *iodide* (333) (829 mg, 79%) as a colourless syrup (Found : C, 50.9; H, 4.1. $C_{28}H_{27}O_9SI$ requires C, 50.5; H, 4.1%); $[\alpha]_D$ - 58.6° (*c* 0.71 in $CHCl_3$); υ_{max} (film) 3035, and 3009 (unsat. CH), 2925, and 2891 (sat. CH), 1745 (C=O), 1615, 1465, 1280, 1189, 1108, and 727 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.37 (3H, s, ArCH_3), 3.40 (1H, dd, $J_{6,5}$ 5.3 Hz and $J_{6,6'}$ - 10.7 Hz, 6-H), 3.43 (3H, s, OCH_3), 3.62 (1H, dd, $J_{6',5}$ 7.7 Hz and $J_{6',6}$ - 10.7 Hz, 6'-H), 4.20 (1H, m, 5-H), 4.24 (1H, d, $J_{1,1'}$ - 10.5 Hz, 1-H), 4.30 (1H, d, $J_{1',1}$ - 10.5 Hz, 1'-H), 5.67 (1H, dd, $J_{4,5}$ 5.6 Hz and $J_{4,3}$ 6.6 Hz, 4-H), 5.77 (1H, d, $J_{3,4}$ 6.6 Hz, 3-H), and 7.25-8.05 (14H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 5.58 (t, C-6), 21.60 (q, ArCH_3), 50.97 (q, OCH_3), 67.66 (t, C-1), 77.55, 79.08, and 80.37 (3d, C-3, C-4 and C-5), 102.56 (s, C-2), 128.05, 128.41, 128.51, 128.77, 128.90, 129.93, 132.37, 133.41, and 133.63 (10d, Ar), 144.95 (s, Ar), 165.13, and 165.68 (2s, C=O); m/z (+ve FAB) 634

(MH^+ - OCH_3, 24%), 539 (MH^+ - HI, 2), 512 (2), and 481 (2).

Methyl 3,4,-di-O-benzoyl-6-deoxy-6-diethylphosphonate-1-O-p-toluenesulphonyl-β-D-fructofuranose (334)

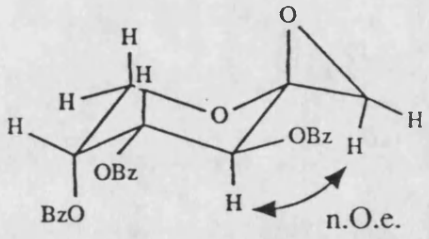
A solution of the iodide (333) (208 mg, 0.31 mmol) in diethyl trimethylsilyl phosphite (6 ml) was heated at 140°C for 48h under nitrogen. The reaction mixture was poured into diethyl ether (100 cm^3), washed with saturated aqueous sodium bicarbonate (2 x 100 cm^3), and saturated brine (100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (from 1:4 to 7:3) as the eluant yielded the *phosphonate* (334) (44 mg, 21%) as a colourless syrup; $[\alpha]_D$ - 61.8° (*c* 0.25 in $CHCl_3$); υ_{max} (film) 2966 and 2916 (sat. CH), 1727 (C=O), 1370, 1269, 1174, 1110, 1019, and 712 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 1.24-1.33 (6H, m, 2 x OCH_2CH_3), 2.25-2.55 (2H, m, 6-H_2), 2.37 (3H, s, $ArCH_3$), 3.47 (3H, s, OCH_3), 4.09 (4H, m, 2 x OCH_2CH_3), 4.19 (1H, d, $J_{1,1'}$ - 10.4 Hz, 1-H), 4.27 (1H, d, $J_{1',1}$ - 10.4 Hz, 1′-H), 4.50 (1H, m, 5-H), 5.71 (2H, m, 3-H and 4-H), and 7.25-8.04 (14H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 16.32 (q, OCH_2CH_3), 16.41 (q, OCH_2CH_3), 21.63 (q, $ArCH_3$), 31.92 (dt, $J_{C,P}$ 141.0 Hz, C-6), 50.18 (q, OCH_3), 61.84 (dt, $J_{C,P}$ 6.7 Hz, OCH_2CH_3), 62.11 (dt, $J_{C,P}$ 4.4 Hz, OCH_2CH_3), 67.53 (t, C-1), 76.19, and 77.13 (2d, C-3 and C-4), 80.07 (dd, $J_{C,P}$ 15.4 Hz, C-5), 102.69 (s, C-2), 127.02, 128.09, 128.41, 128.48, 128.80, 128.96, 129.06, 129.84, 129.94, 130.91, 132.37, 133.27, and 133.57 (13d, Ar), 144.95 (s, Ar), 165.26, and 165.71 (2s, C=O); m/z (+ve FAB) 677 (MH^+, 34%), 645 (MH^+ - CH_3OH, 83), and 523 (MH^+ - CH_3OH - $C_6H_5CO_2H$, 10).

Methyl 3,4,5-tri-O-benzoyl-1-O-p-toluenesulphonyl-β-D-fructofuranose (336)

A solution of the di-tosylate (332) (347 mg, 0.49 mmol) and sodium benzoate (85 mg, 0.59 mmol) in dry DMF (10 cm^3) was heated at 90°C for 16h under nitrogen. The reaction mixture was poured into diethyl ether (100 cm^3), washed with saturated brine (3 x 100 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel with ethyl acetate-light petroleum (b.p. 60-80°C) (1:4) as the eluant yielded the *tri-benzoate* (336) (294 mg, 99%) as a colourless syrup (Found :C, 63.4; H, 4.8. $C_{35}H_{32}O_{11}S$ requires C, 63.6; H, 4.9%); $[\alpha]_D$ - 43.3° (*c* 1.01 in $CHCl_3$); υ_{max} ($CHCl_3$) 2912 (sat. CH), 1734 (C=O), 1347, 1238, 1180, 1078, and 975 cm^{-1}; δ_H (270 MHz, $CDCl_3$) 2.37 (3H, s, ArCH_3), 3.36 (3H, s, OCH_3), 4.25 (1H, d, $J_{1,1'}$ - 10.4 Hz, 1-H), 4.30 (1H, d, $J_{1',1}$ - 10.4 Hz, 1'-H), 4.43 (1H, m, 5-H), 4.55 (1H, dd, $J_{6,5}$ 5.9 Hz and $J_{6,6'}$ - 12.0 Hz, 6-H), 4.70 (1H, dd, $J_{6',5}$ 3.9 Hz and $J_{6',6}$ - 12.0 Hz, 6'-H), 5.85 (1H, d, $J_{3,4}$ 7.0 Hz, 3-H), 5.92 (1H, dd, $J_{4,5}$ 6.6 Hz and $J_{4,3}$ 7.0 Hz, 4-H), and 7.24-8.08 (19H, m, Ar*H*); δ_C (67.8 MHz, $CDCl_3$) 21.57 (q, ArCH_3), 50.11 (q, OCH_3), 64.25, and 67.95 (2t, C-1 and C-6), 76.09, 76.51, and 78.30 (3d, C-3, C-4 and C-5), 102.43 (s, C-2), 127.99, 128.35, 128.44, 128.70, 128.86, 129.51, 129.64, 129.74, 129.90, 132.40, 133.15, 133.37 and 133.57 (13d, Ar), 144.92 (s, Ar), 165.25, 165.65, and 166.00 (3s, C=O); m/z (CI) 629 (MH^+ - CH_3OH, 47%), 475 (MH^+ - $CH_3C_6H_4O_2SOCH_3$, 35), and 391 (53).

APPENDIX 1

270 MHz ^{1}H spectra, and n.O.e. experiments, of (267).



$^{3}J_{3,4}$ 9.9Hz

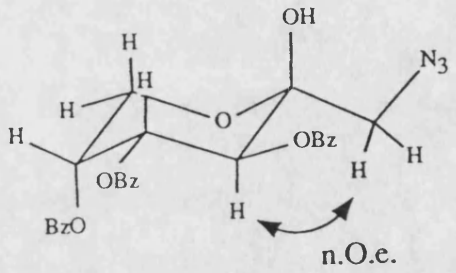
(267)

The n.O.e. enhancement observed was not quantified, but this was used as supporting evidence for the structural assignment that was made.


GD55/2.NOE.2.89pm.
PPM
8
7
6
5
4
3
2
1

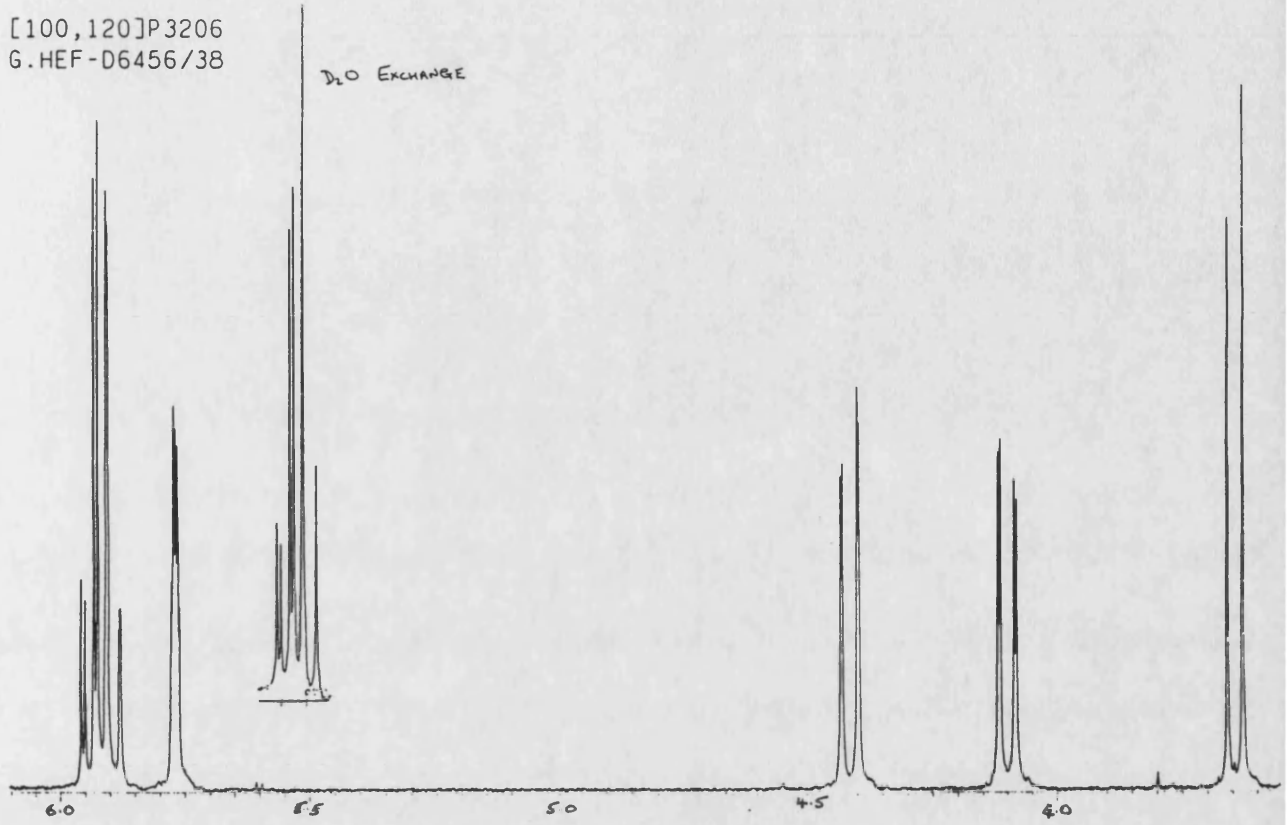
APPENDIX 2

400 MHz ^1H spectra, and n.O.e. experiments, of (271).

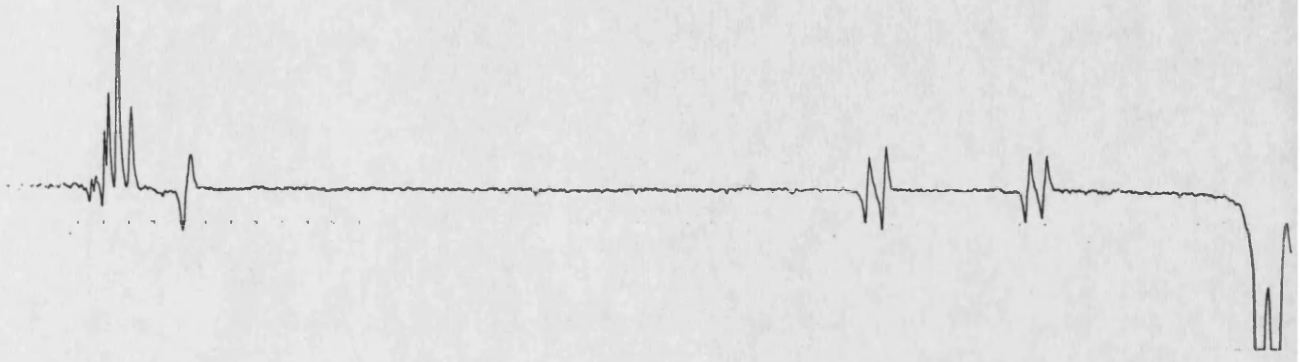


(271) $^3J_{3,4}$ 9.8Hz

The n.O.e. enhancement observed was not quantified, but this was used as supporting evidence for the structural assignment that was made.

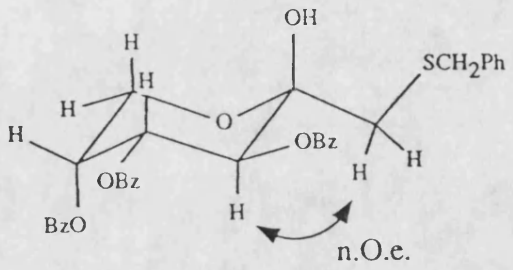


[100,120]P3206NOE1
G.HEF-D6456/38



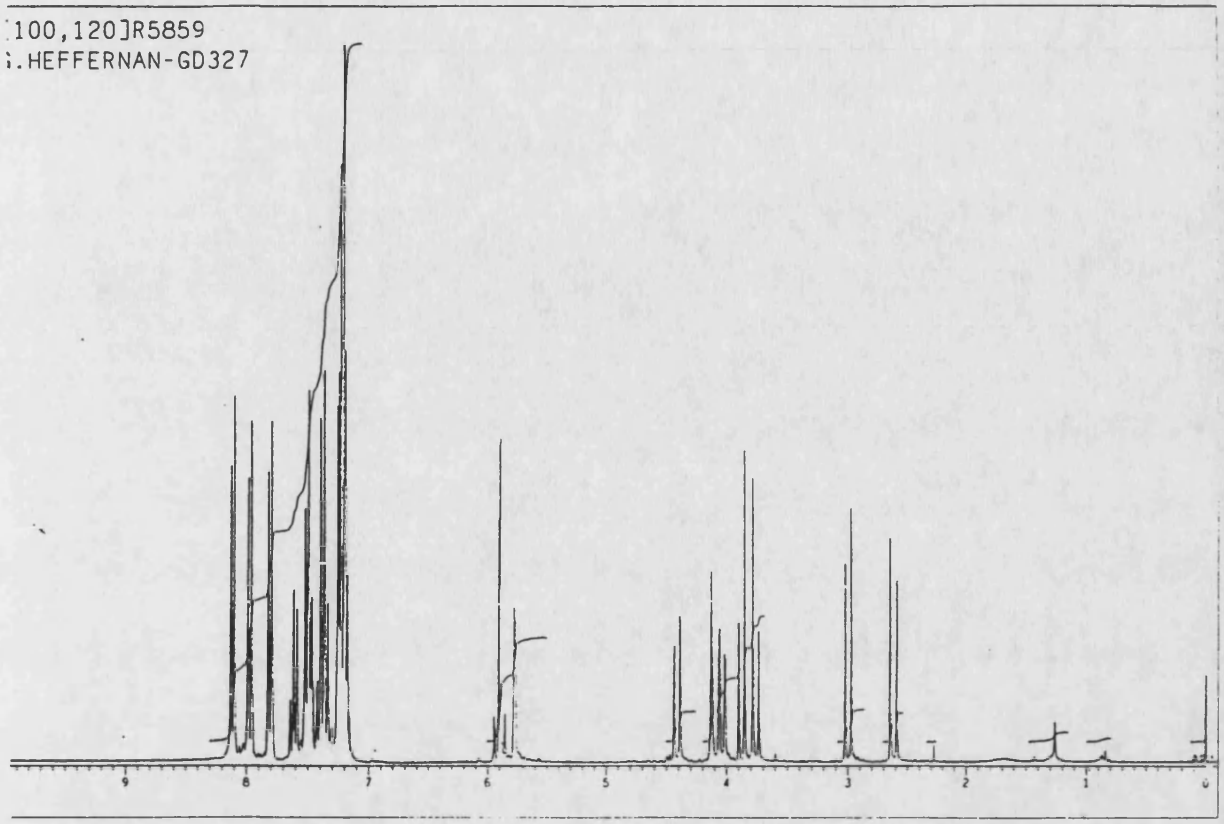
APPENDIX 3

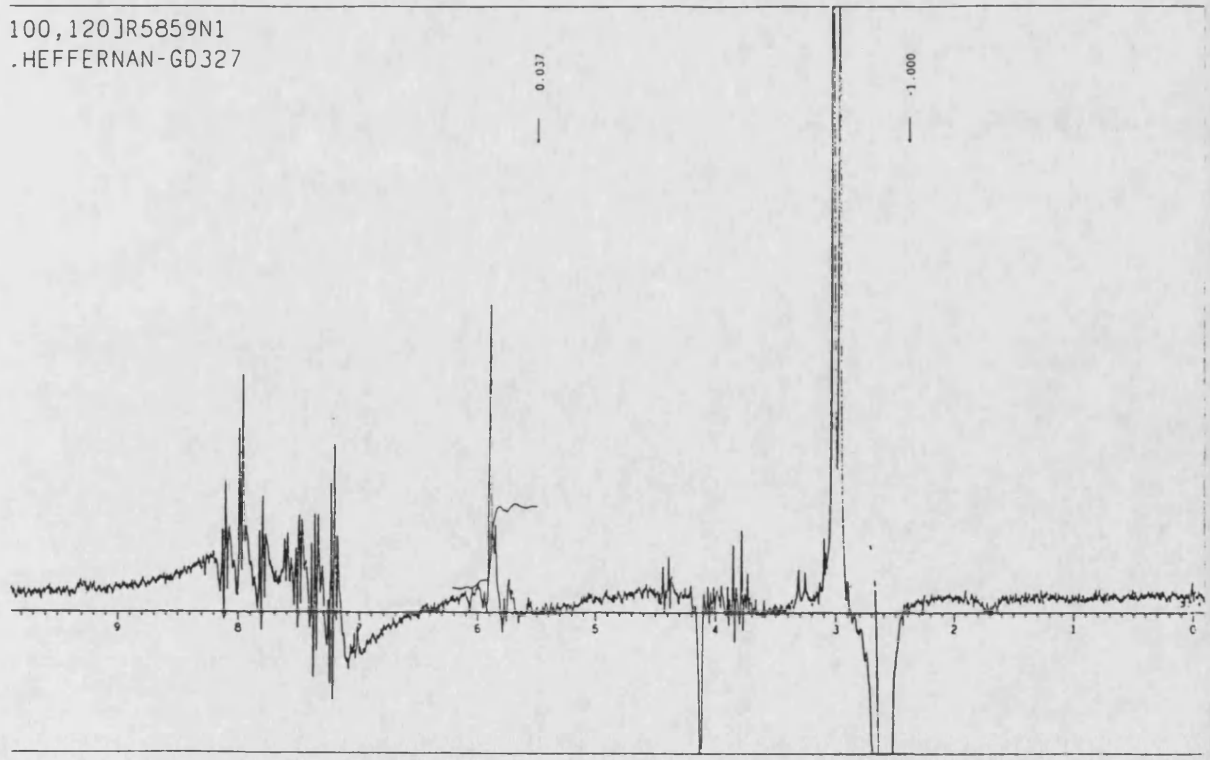
400 MHz ^1H spectra, and n.O.e. experiments, of (272).



(272) $^3J_{3,4}$ 10.4Hz

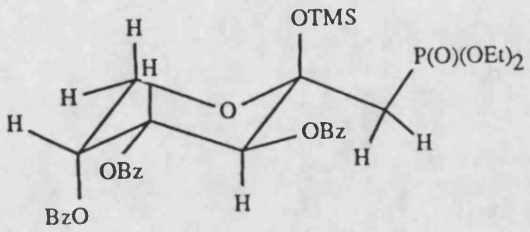
The n.O.e. enhancement observed was not quantified, but this was used as supporting evidence for the structural assignment that was made.


100,120]R5859
.HEFFERNAN-GD327

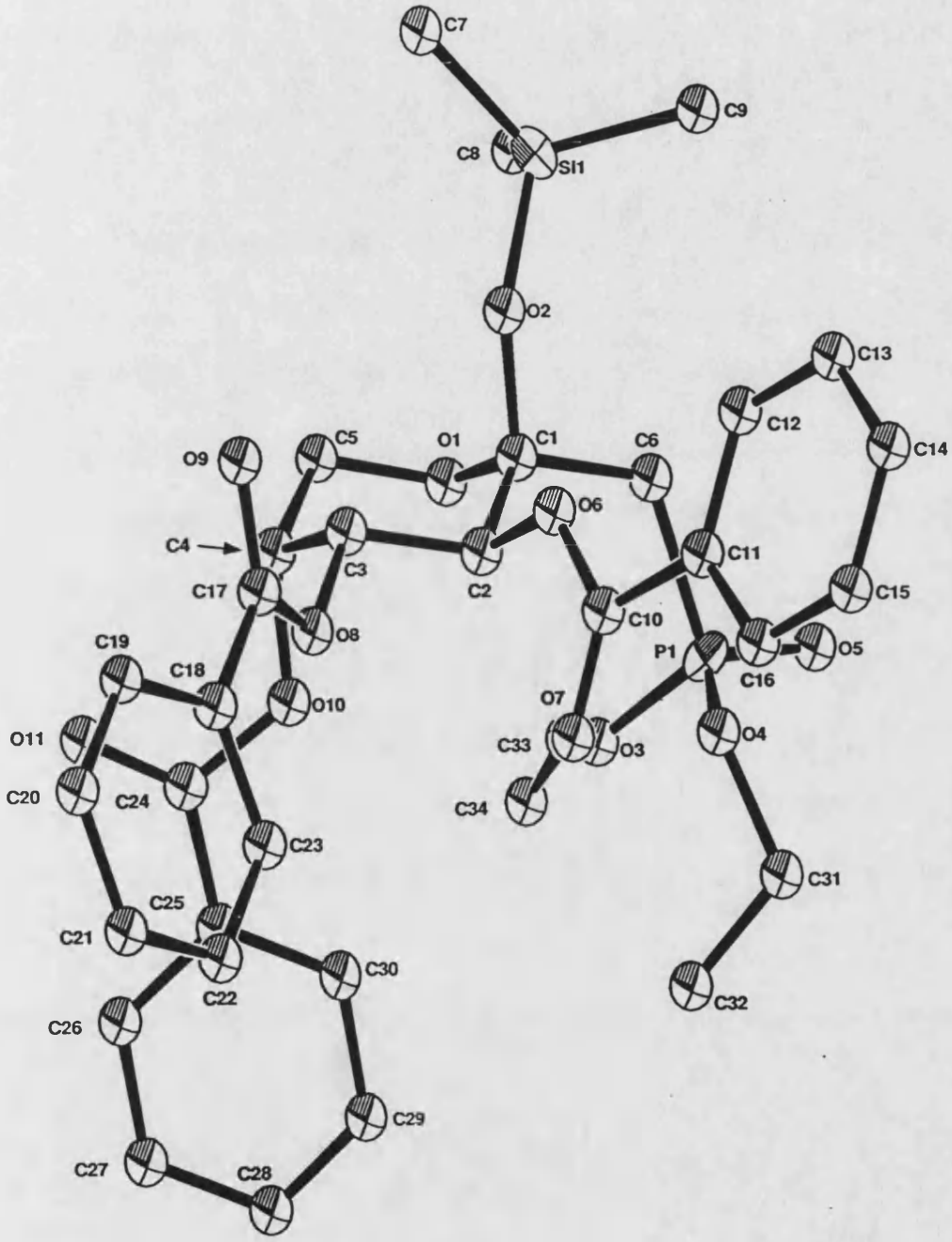

100,120]R5859N1
.HEFFERNAN-GD327
0.037
1.000

APPENDIX 4

ORTEP diagram resulting from a single crystal X-ray diffraction experiment on (285).

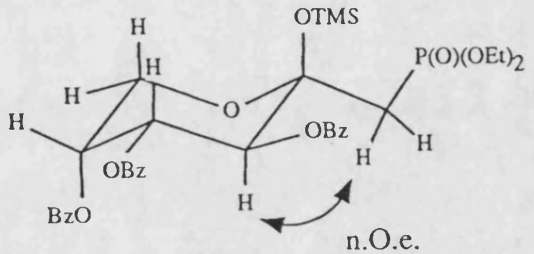


(285)


C7
C9
C8
Si1
O2
C13
C12
C14
C5
O1
C1
C6
O9
O6
C4
C3
C2
C11
C17
C15
C10
O8
C19
P1
O5
C18
C16
O10
O7
O11
C33
O3
O4
C20
C24
C34
C23
C31
C25
C21
C30
C22
C32
C26
C29
C27
C28

APPENDIX 5

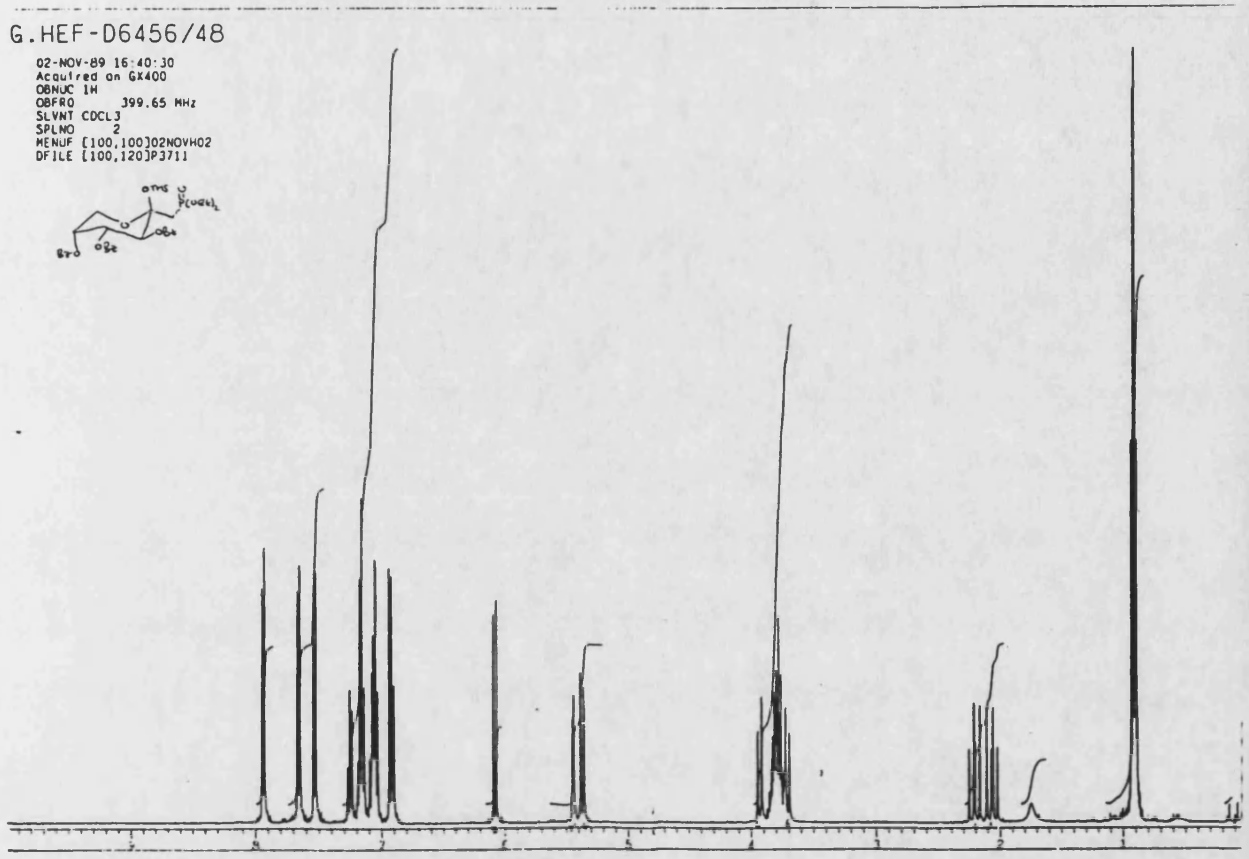
400 MHz ^1H spectra, and n.O.e. experiments, of (285).

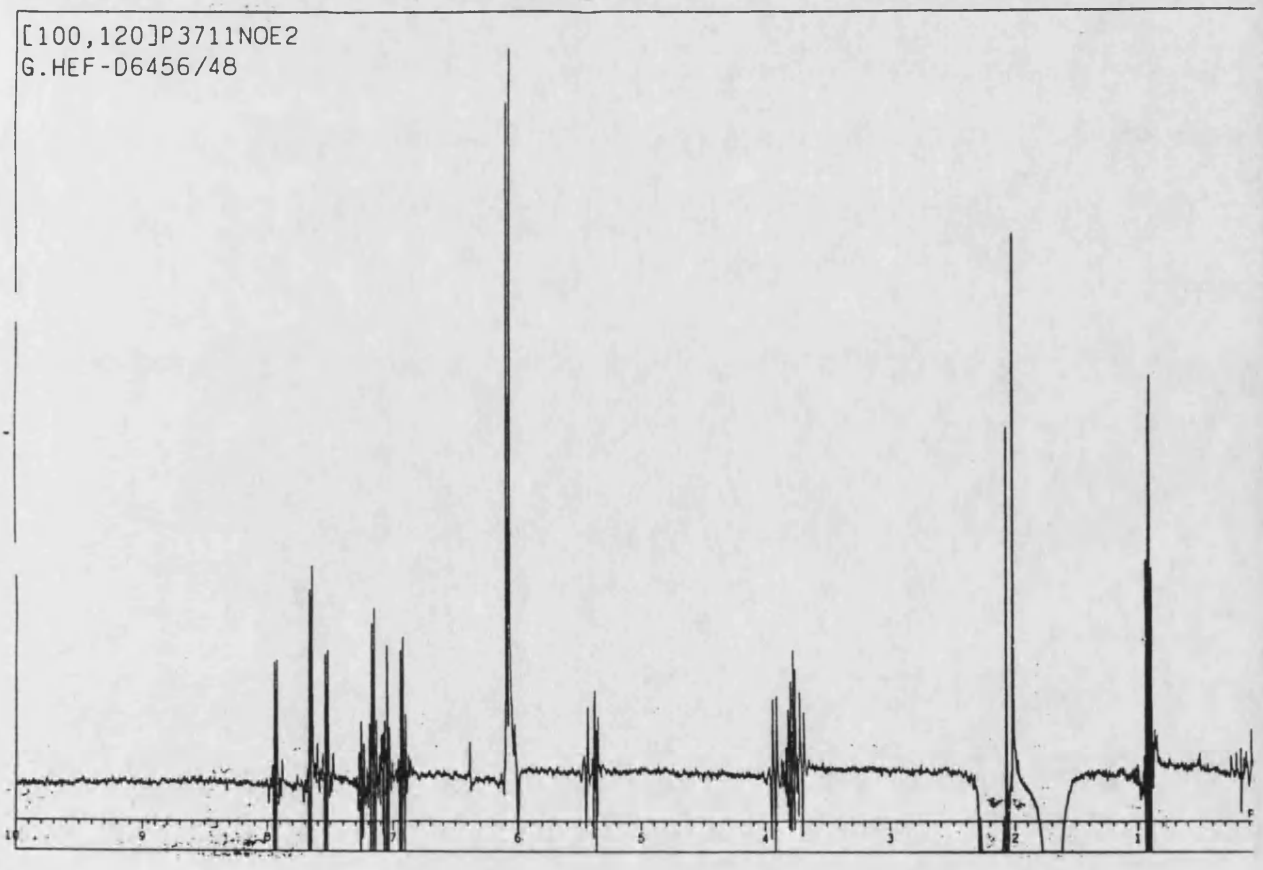


$^3J_{3,4}$ 10.1Hz

(285)

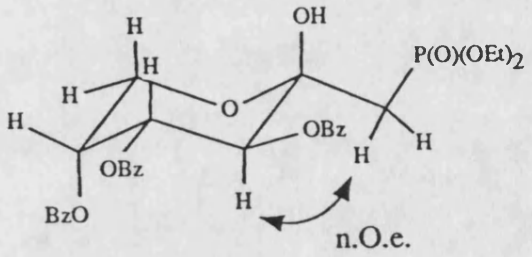
The n.O.e. enhancement observed was not quantified, but this was used as supporting evidence for the structural assignment that was made.


G.HEF-D6456/48
02-NOV-89 16:40:30
Acquired on GX400
OBNUC 1H
OBFRQ 399.65 MHz
SLVNT CDCL3
SPLNO 2
MENUF [100,100]02NOVH02
DFILE [100,120]P3711


[100,120]P3711NOE2
G.HEF-D6456/48

APPENDIX 6

400 MHz ^1H spectra, and n.O.e. experiments, of (283).



$^3J_{3,4}$ 10.4Hz

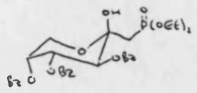
(283)

The n.O.e. enhancement observed was not quantified, but this was used as supporting evidence for the structural assignment that was made.

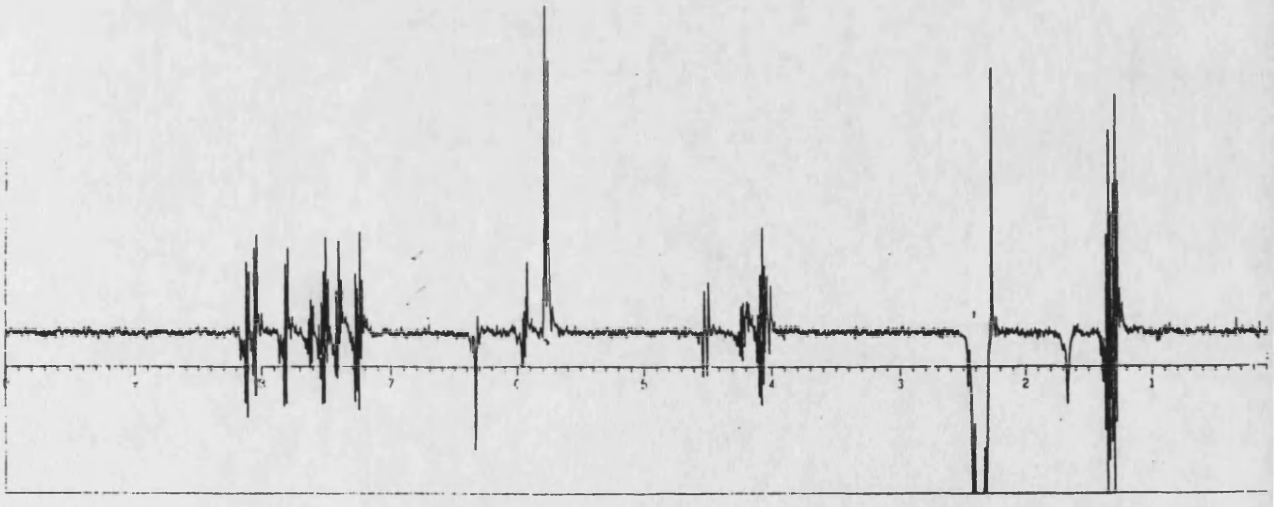
G.HEF-D6456/46-2

02-NOV-89 16:32:52
Acquired on GX400
OBNUC 1H
OBFRQ 399.65 MHz
SLVNT CDCL3
SPLNO 1
MENUF U2NOVH01
DFILE [100,120]P3570

GD 180/2

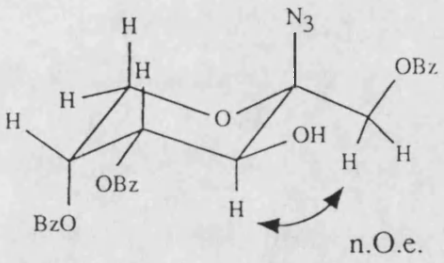


[100,120]P3570NOE1
G.HEF-D6456/46-2



APPENDIX 7

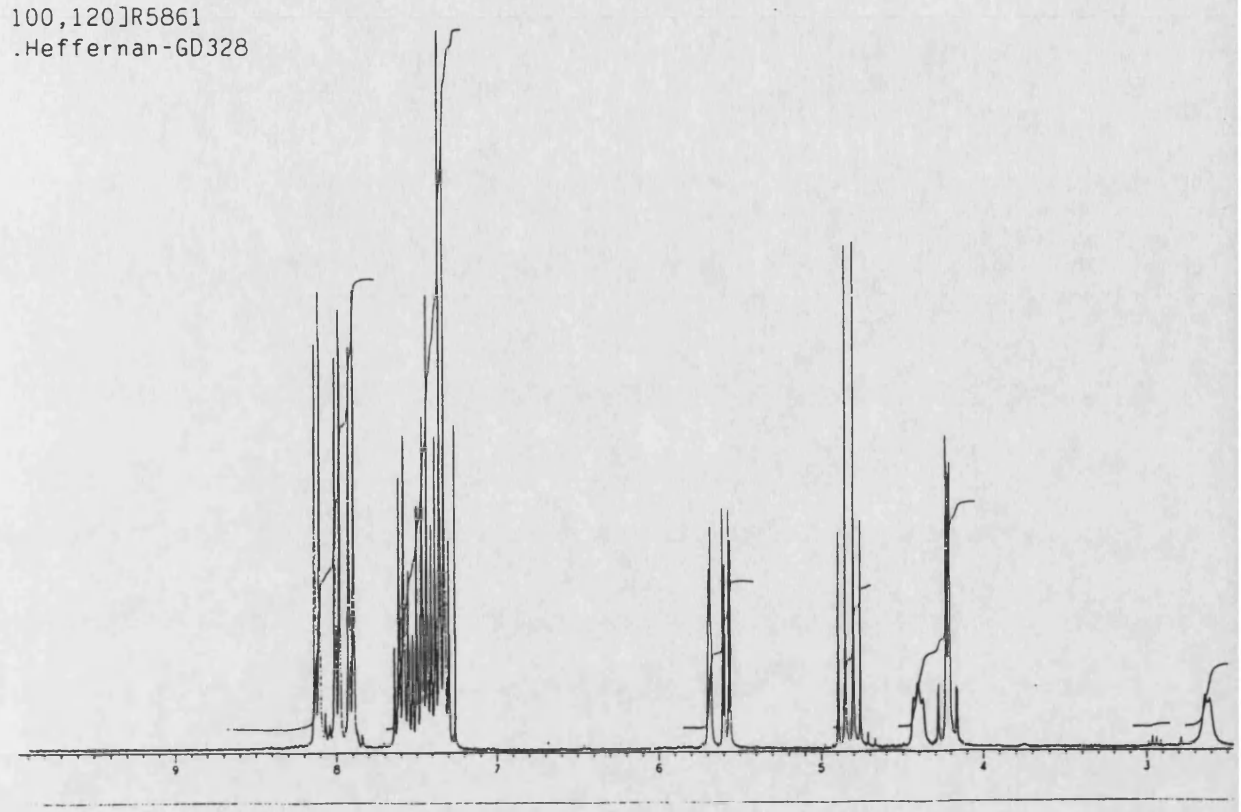
400 MHz ^{1}H spectra, and n.O.e. experiments, of (289).

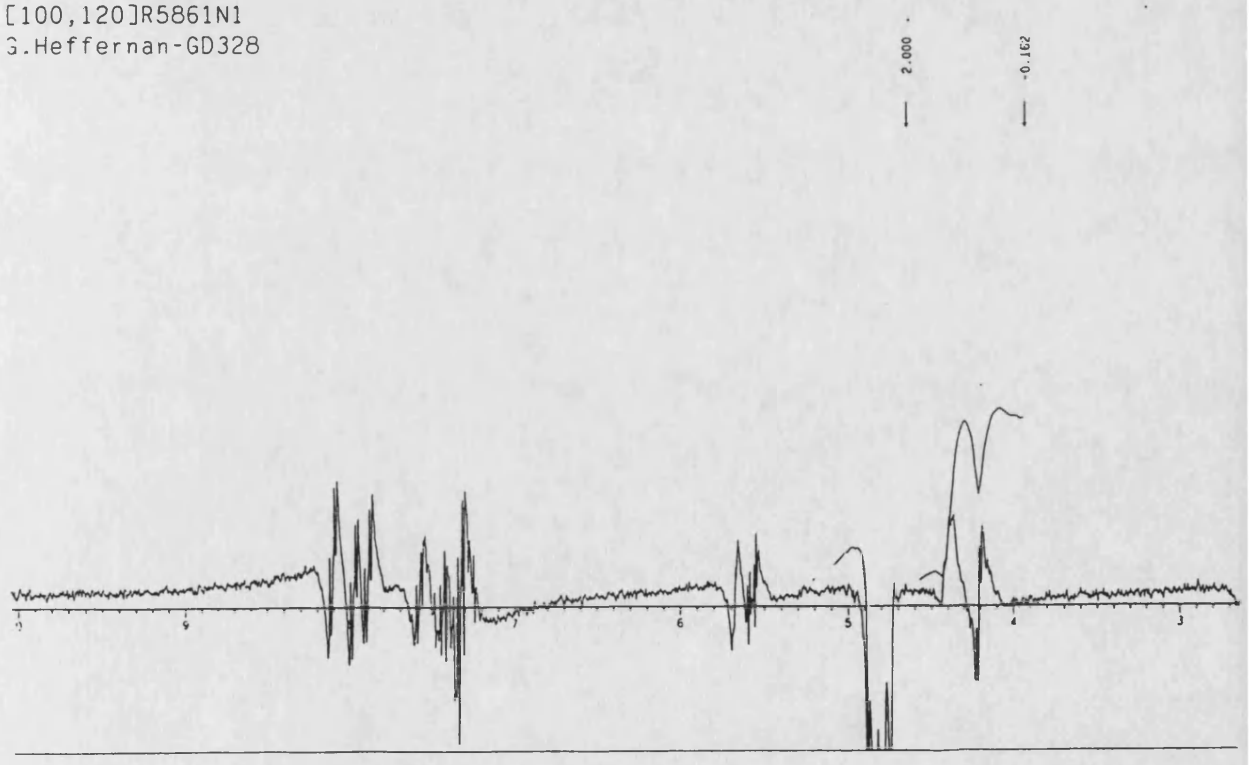


$^{3}J_{3,4}$ 10.1Hz

(289)

The n.O.e. enhancement observed was not quantified, but this was used as supporting evidence for the structural assignment that was made.


100,120]R5861
.Heffernan-GD328


[100,120]R5861N1
3.Heffernan-GD328
2.000
-0.162

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